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ENGINEERING AND DEVELOPMENT SUPPORT OF GENERAL DECON TECHNOLOGY FOR THE U.S. ARMY'S INSTALLATION RESTORATION PROGRAM

Task 11. Composting of Explosives

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The primary objective of this study was to determine the extent to which TNT and RDX concentrations are reduced by composting under controlled conditions in the laboratory over a period of six weeks. A second objective was to determine if bench-scale composting studies accurately simulate the activity of a larger-scale compost by comparison of parallel studies monitoring TNT and RDX disappearance in laboratory scale (50 g dry weight) and greenhouse (10 kg dry weight) composts. An additional objective was to determine the leachability of TNT or RDX from compost.

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Uniformly ring labeled ^{14}C-TNT or ^{14}C-RDX were used in the laboratory studies. A 50% reduction in TNT concentrations was demonstrated after three weeks of composting with a total reduction of 82.6% at the end of six weeks. No significant quantities of $^{14}CO_2$ were evolved indicating that composting did not result in cleavage of the ring structure of the TNT molecule. Reduction products normally formed from aerobic transformation of TNT were not detected after three weeks of composting. Trace quantities of 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene were found in one of three replicate composts after six weeks of composting. The RDX laboratory composts showed a reduction in the RDX concentration of 31.2% after three weeks of composting and a total reduction of 78.3% after six weeks of composting. Significant amounts of $^{14}CO_2$ were produced by the RDX compost indicating that cleavage of the RDX molecule occurred during composting.

The greenhouse compost studies demonstrated a very rapid decrease in the TNT concentration. At the three week sampling time, the TNT concentration had been reduced by 99.9%. Analysis of the four week TNT compost extract confirmed that the TNT concentration in the composted material was below the detection limit of 16.9 ppm. Greenhouse composting of RDX resulted in a 61% reduction in the RDX concentration after three weeks with a total reduction of 82% following six weeks of composting.

SUMMARY

The objective of this study was to determine the extent to which TNT and RDX concentrations are reduced by composting under controlled conditions in the laboratory over a period of six weeks. A second objective was to determine if bench-scale composting studies accurately simulate the activity of larger composts by comparison of parallel studies monitoring TNT and RDX disappearance in laboratory scale (50 g dry weight) and greenhouse (10 kg dry weight) composts. An additonal objective was to determine the leachability of TNT or RDX from compost.

A portion of the explosives used in the laboratory studies contained a ^{14}C-tracer (^{14}C-TNT or uniformly ring labeled ^{14}C-RDX). Each explosive was added to an initial concentration of 1% in the composts. Composts (50 g dry weight) were incubated at 55°C with continuous aeration. Offgases were monitored for $^{14}CO_2$, volatile ^{14}C-amines and other volatile ^{14}C-organics. Composted material was solvent extracted after three and six weeks of composting. Extracts were monitored by liquid scintillation counting for ^{14}C-activity. Thin layer chromatography and autoradiography were used to determine the portion of the radioactivity present in the extract as the parent molecule and to isolate ^{14}C-containing solvent extractable products from composting of the ^{14}C-labeled explosives.

Greenhouse scale composts (10 kg dry weight) contained production grade TNT (2% by weight) or RDX (1%) by weight and composted for four to six weeks. Aerobic conditions were maintained in these composts by a forced aeration system and by frequent mixing. No external energy was supplied to heat these composts. Each compost was sampled after three weeks of composting and after four or six weeks of composting. The samples were extracted and the extracts were analyzed by gas chromatography to determine the concentration of explosives remaining in the compost material.

Composting under laboratory conditions resulted in a decrease in the TNT concentration of 50% after three weeks and a reduction of 82.6% at the end of six weeks. Significant quantities of $^{14}CO_2$ were not evolved by these composts indicating that cleavage of the ring structure did not occur during composting. TNT reduction products usually formed in the biotransformation of TNT were not detected after three weeks of composting. Trace quantities of 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene were found in one of three replicate composts after six weeks of composting.

RDX concentrations were reduced by 31.2% after composting for three weeks under controlled laboratory conditions. A reduction of 78.3% in the RDX concentration was demonstrated after six weeks of composting. $^{14}CO_2$ was produced by these composts in significant amounts indicating that cleavage of the RDX molecule occurred during composting.

A very rapid decrease in TNT concentration was demonstrated in the greenhouse compost studies. After three weeks of composting, the initial TNT concentration of 20,000 ppm had been reduced by 99.9%. Analysis of the four week TNT compost extract confirmed that the TNT concentration in the composted material was below the detection limit. Breakdown of RDX in the greenhouse compost was initially more rapid than in laboratory composts. After three weeks of composting, RDX levels in the greenhouse composts were reduced by 61%. Total reduction of RDX by composting for six weeks averaged 82%.

Results from the laboratory and greenhouse composts indicate that both RDX and TNT concentrations are rapidly decreased by composting. Explosives levels are reduced by 80% or more within six weeks. Data from laboratory composting in these studies provided a good estimate of the breakdown of explosives in larger scale composts.

The leachate study was performed under conditions designed to illustrate a "worst case" example. The soil used in the study was selected to have a relatively low capacity to absorb and retain organics such as TNT or RDX and the 24-hour extraction would likely result in TNT and RDX concentrations far greater than would be found following rainfall and leaching from an outdoor compost pile. Analysis of the RDX compost leachate at time zero showed that 7.4% of the RDX (approximately 124 ppm) was leached into the water extract. A significant decrease in RDX content was observed in the 3 week compost leachate (52.5 ppm) and in the 6 week compost leachate (13 ppm). The decrease in the RDX concentration in the leachates corresponds to the biodegradation of this explosive during the composting period. Analysis of the TNT compost leachate showed that TNT was not leached into the water extract in detectable amounts from fresh compost materials. The three-week TNT compost leachate contained 98 ppm TNT and the six-week TNT compost leachate contained 1.4 ppm TNT. These results indicate that adsorption of TNT to compost materials is altered during composting to allow increased leaching of TNT into the extract by three weeks. The subsequent decrease in TNT concentrations in the 6-week leachate corresponds to the disappearance of TNT during the composting period.

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I. INTRODUCTION

A. Background

The manufacture and handling of explosives such as TNT and RDX has resulted in contamination of soils and sediments in areas where these activities have taken place over extended periods of time. In general, the concentrations of RDX and TNT in soils are in the low ppm range. Lagoon sediments, however, contain large concentrations of these explosives, i.e. up to 10% by weight. These lagoons have been used for wastewater disposal from shell loading and cleaning operations and, although the wastewaters generally contain less than 100 mg/L of the explosives, over the years the explosives have precipitated out of the water and collected in the sediment of the lagoons.

In a review of the literature to evaluate biological degradation of explosives as a potential cost-effective method for decontamination of soils and sediments, it was found that microbial degradation of RDX proceeds slowly or not at all under aerobic conditions. Rapid degradation of RDX does occur under anaerobic conditions. TNT is biotransformed by microorganisms under aerobic conditions, but no evidence for biodegradation (ring cleavage) was reported. Biotransformation of TNT results in a variety of reduction products. Some of these products are environmentally unacceptable. The literature review identified composting as a biological method with potential for low-cost decontamination of soils and sediments.

Composting is a process of controlled biological degradation in which almost any degradable organic substance may be converted through microbial activity to a product with the general appearance and many of the characteristics of a fertile soil. The compost environment is radically different from that found in aerobic soil and sediments because of the elevated temperatures and the variations in active microbial populations. Mesophilic organisms thrive when compost tmeperatures range from normal ambient temperatures to 45°C. When the compost temperature exceeds 45°C, thermophilic organisms proliferate and tolerate relatively high temperatures. Historically compost has been used in agriculture to convert organic wastes into a product useful as fertilizer and/or soil conditioner. Composting can occur over a wide range of conditions in which a natural biological process is stimulated to decompose complex organic molecules into simpler compounds through the growth and activity of bacteria, actinomycetes and fungi. The microorganisms use a portion of the carbon and nitrogen in the compost materials for synthesis of microbial biomass and convert chemical energy into heat through respiration. The heat produced increases the temperature of the composting mass and evaporates moisture. Composting can occur in an aerobic mode over a wide range of moisture contents. The moisture content must be at least 35% for optimal composting although excessive moisture may result in displacement of air from pore spaces by water and may lead to anaerobic conditions. Accelerated aerobic composting can be achieved by forced aeration in which compost materials are mixed and bulking materials are added as needed.

Composting as a technique for disposal of hazardous materials in soils and sediments is applicable in almost any environment. In situ composting requires only bulk materials (to provide sufficient pore space for aeration), proteinaceous material (for carbon and nitrogen sources), water and air; materials which are readily available in almost any locale. Composting for decontamination of soil or sediment is relatively easy. The soil or sediment is thoroughly mixed with the compost materials. The maximum amount of soil in the mixture will depend on the concentration of the hazardous material in the soil and the type of soil. The concentration of hazardous material must not be so high as to inhibit the growth of the microbial populations. The type of soil can also influence the effectiveness of the compost in degrading hazardous materials. The texture and the organic content of the soil will determine how readily the soil disperses in the compost. Ideally the soil thinly coats the organic bulk of the compost, thus exposing individual particles and small aggregates of soil to the microbial populations. Soils with high clay and/or organic matter contents may be relatively sticky and tend to clump rather than disperse. The absorptive properties of the the soil may present an additional complication, i.e. the soil may bond the hazardous materials strongly enough to inhibit microbial attack. The interaction between soil, the hazardous material, and the microbial population is difficult to predict; however, in most situations it is not expected to significantly retard degradation of the hazardous waste. Contaminated water could also be decontaminated in this system by using this water as the source of moisture for the compost pile.

This report presents the results of laboratory-scale and greenhouse scale composting experiments for decontamination of soils contaminated with TNT or RDX. The report is organized in the following manner. The basic materials used in the study and the analytical methods are discussed in Section II. Section III presents the Preliminary TNT Laboratory Compost. Laboratory Composting of TNT and RDX and Greenhouse Composting are presented in Sections IV and V, respectively. The final section (VI) contains the Leachate Study.

B. Objectives

The primary objective of this study, Composting of Explosives, was to determine the extent to which TNT and RDX concentrations are reduced by composting under controlled conditions in the laboratory over a period of six weeks. A second objective was to determine if bench-scale composting studies accurately simulate the activity of a larger-scale compost by comparison of parallel studies monitoring TNT and RDX disappearance in laboratory scale (50 g dry weight) and greenhouse (10 Kg dry weight) composts. An additional objective was to determine the leachability of TNT or RDX from the compost. Identification of the breakdown products of TNT and RDX under compost conditions and evaluation of the toxicity of the products or leachates were not within the scope of this task.

II. MATERIALS, EQUIPMENT AND ANALYTICAL METHODS

A. Equipment

The following major pieces of equipment were utilized in this study:

- Hewlett-Packard 5880A Gas Chromatograph with electron capture detector, computer controller, integrator and autosampler
- Varian 3700 Gas Chromatograph with thermoconductivity detector, computer controller, and integrator
- Beckman LS7500 Liquid Scintillation Counter
- Greenhouse with temperature control
- Incubator, 55°C
- Water bath, 37°C
- Ball Mill
- Virtis Lyophylizer
- Hoskins Electric Furnace

B. Compost Materials

1. Carbon and Nitrogen Source

The composts used in these studies were primarily composed of a 50:50 (by weight) mixture of hay and horse feed. Alfalfa was selected as the hay to be used because of its high leaf to stem ratio and its high protein content. Baled alfalfa hay was obtained and chopped into segments 40 cm (1.6 inches) or less. The horse feed used was Purina Sweetena. This feed appeared to contain cracked corn, oats, finely ground pelletized hay and molasses. The nitrogen content of both the hay and horse feed was sufficiently high so as not to limit microbial activity.

2. Seed Compost

An alfalfa hay - Purina horse feed compost was maintained in an active state to supply microorganisms to seed into freshly started laboratory and greenhouse composts. This compost was initiated with a small quantity of sewage sludge as a seed. As the readily available nutrients in this compost were depleted, a fraction of the compost was disposed of and additional hay and horse feed were added. This compost was aerated by mixing every 1 to 3 days.

3. Soil

Soil was used as a carrier to mix the explosives into the compost. The soil used was a Lakeland sand. Prior to use, the soil was air dried and sieved (2 mm) to remove pebbles, rocks, and large pieces of plant material. Some physical and chemical analyses of this soil are as follows:

% sand	95.1
% silt	3.0
% clay	1.9
% organic matter[1]	1.0
pH[1]	6.7

A soil composed primarily of sand with a low organic matter content would not be expected to bind to or strongly interact with, TNT or RDX. This soil was selected to minimize the possible effects of adsorption on the availability of RDX and TNT for microbial attack in the compost.

4. TNT AND RDX

The TNT and RDX used to spike the composts were production grade explosives. Near saturated solutions of TNT and RDX were maintained in acetone as a stock for addition to compost. The stock was protected from light and stored at ambient temperatures. TNT or RDX concentrations were determined by diluting a subsample of the stock for analysis by gas chromatography (GC). The analytical methods are described in Section IIB. No attempt was made to characterize impurities or examine their metabolism in compost.

5. ^{14}C-Labeled Explosives

The purity of ^{14}C-labeled TNT and RDX was determined by thin layer chromatography and autoradiography. All spots on the chromatograph, spots identified by radiography, visible spots and spots visible under ultraviolet light, were scraped into vials for liquid scintillation counting.

Uniformly ring labeled ^{14}C-TNT was obtained from Pathfinder Laboratories. Purity of the ^{14}C-label was determined by developing separate chromatographs in two solvent systems. The results are given in Table 1. Using benzene:toluene:hexanes (10:10:5) as a solvent system, 96.1% of the ^{14}C activity was associated with TNT. A second solvent system [benzene:hexanes: pentane:acetone (50:40:10:3)] was found to be superior to the first system in that it separated out a larger number of compounds. This chromatograph indicated that 92% of the ^{14}C was incorporated into TNT. Trace amounts of ^{14}C-labeled 2,2',6,6'-tetranitro-4,4'-azoxytoluene or closely related compounds may have been present in the stock solution. The mono- or diamino derivatives of TNT were not detected.

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[1]Analysis performed by the Soil Testing and Plant Analysis Laboratory, Virginia Polytechnic Institute and State University.

Table 1. Results of Thin Layer Chromatographic Analysis of ^{14}C-TNT Stock

	R_f	Probable Compound (R_f)	DPM	Percent of Total
Benzene:Toluene:Hexanes (10:10:5)	0.59	TNT (0.52)	132459	96.1
	0.52	-	2370	1.7
	0.44	Tetra (0.39)	1887	1.4
	0.00	-	1184	0.9
Benzene:Hexanes:Pentane:Acetone (50:40:10:3)	0.47	-	303	0.3
	0.41	TNT (0.38)	77744	92.0
	0.34	-	5722	6.3
	0.29	Tetra (0.30)	438	0.5
	0.09	-	190	0.2
	0.04	-	247	0.3
	0.00	-	352	0.4

Uniformly ring labeled ^{14}C-RDX was synthesized by Atlantic Research Corporation. The source of ^{14}C used to make RDX was ^{14}C-formaldehyde purchased from Pathfinder Laboratories. The method of synthesis is given in Appendix A.

The thin layer chromatograph of ^{14}C RDX was developed in a 4:1 mixture of methylene chloride and acetonitrile. The radiochemical purity of RDX was high, with the RDX containing 97.0% of the radioactivity. Additional activity was located at the origin on the chromatograph and in an unidentified spot with an R_f of 0.49. The results are summarized in Table 2.

C. Analytical Methodology

1. Development of Procedures to Extract TNT from Compost

a. Cold Acetone Extraction

Chopped alfalfa and horse feed material (50 g dry weight) were spiked at 10,000 ppm TNT containing 0.25 Ci ^{14}C-TNT and extracted 3X with 400 mL acetone followed by two x 400 mL benzene extractions.

Sample A - 41.7% recovery in acetone extract
4.1% recovery in benzene extract

Sample B - 42.0% recovery in acetone extract
3.9% recovery in benzene extract

b. Cold Acetone Extraction With Agitation

Seven week old material (50 g dry weight) was spiked at 10,000 ppm TNT containing 0.24 μCi ^{14}C-TNT and extracted with 400 mL acetone on a shaking table for 30 minutes. The extraction was repeated twice for a total of 3 extractions. The compost was then extracted twice with 400 mL benzene for 30 minutes on a shaking table followed by one extraction with 400 mL distilled water adjusted to a pH of approximately 3 (HCl) for 30 minutes on a shaking table. An additional water extraction was performed with 400 mL distilled water adjusted to a pH of approximately 11 (NaOH):

- 64.8% recovery in acetone
- 13.0% recovery in benzene extract
- No significant recovery was obtained in acidic or basic water extracts

Table 2. Thin Layer Chromatographic Analysis of ^{14}C-RDX Stock

	R_f	Compound (R_f)	DPM	Percent of Total
Methylene chloride:acetonitrile (4:1)	0.72	RDX (0.74)	20168	97.0
	0.49	-	426	2.0
	0.00	-	158	0.8

c. Warm Acetone Extraction

Composted material (50 g dry weight) was spiked at 10,000 ppm TNT containing 0.24 µCi ^{14}C-TNT and extracted with 400 mL acetone at 37°C with agitation for 15 minutes. Two additional warm acetone extractions were performed:

Sample A - 87.9% recovery

Sample B - 89.0% recovery

This procedure was used for preliminary TNT laboratory compost extractions.

d. Benzene/Methanol Extraction for TNT in Compost

One hundred and sixty mL of warm benzene/methanol (120:40) were added to 20 g (dry weight) composted material. These samples were warmed to 37°C in a water bath and agitated by shaking every 5 minutes for 30 minutes. The extract was then filtered by vacuum through Whatman #2 filter paper. The solids were re-extracted twice with 160 mL warm benzene (for a total of 3 extractions). Recovery of ^{14}C from compost samples ranged from 97.9% to 94.3% using this procedure.

2. Development of Procedures to Extract RDX from Compost

One hundred sixty mL of warm acetone were added to 20 g (dry) weight) composted material. These samples were placed in a water bath to maintain a temperature of 37°C. The samples were agitated at 5 minute intervals. After 30 minutes, the extract was filtered by vacuum through Whatman #2 filter paper. The solids were extracted twice with 160 mL warm acetone (for a total of 3 extractions). The extracts from the three extractions were pooled. This extraction procedure resulted in recovery of 97.5% of the ^{14}C-RDX spiked into the composted material.

3. Quantitative Analysis of TNT

Composted material (50 g wet weight) is extracted with 160 mL benzene:methanol (75:25). Warm extractant, 160 mL is added to each jar containing the compost material and the jars are placed in a 37°C waterbath. Jars are agitated at 5 minute intervals. Jars are removed from the waterbath after 30 minutes. The liquid extract from each jar is filtered through Whatman #2 filterpaper into a glass flask. The filtrate is transferred to glass culture tubes and diluted as necessary for analysis by GC.

a. Instrumentation

Gas chromatograph - Hewlett-Packard 5880A with computer controller and integrator, autoinjector and electron capture detector.

b. Parameters

Column - 1.5% OV17/1.95% OV210 on 80/100 Anakrom Q in a 2 mm I.D., 0.125 in. O.D. by 6 ft. glass column.

Temperature: injection port - 210°C
oven - 180°C
detector - 300°C
Temperature Programming - isothermal
Carrier Gas - nitrogen at 28 cc/min.
Detector - electron capture
Injection Volume - 2 µL
Retention Time - 3.2 min.

c. Calculations

The concentration of explosive (ppb) in the sample is read directly from the standard curve. The apparent concentration of explosive in the compost is calculated from the formula given below:

$$\text{Concentration (ppm)} = \text{ppb} \times \frac{\text{120 mL extract x 0.001 x reciprocal of extract dilution}}{\text{g dry weight compost}}$$

4. Quantitative Analysis of RDX

Composted material (50 g wet weight) was weighed into jars and extracted three times with acetone. Warm acetone, 160 mL, is added to each jar containing the composted material and the jars are then placed in a 37°C water bath. All jars are agitated at 0, 10 and 20 minutes. Jars are removed from the water bath after 30 minutes. The liquid extract from each jar is filtered by vacuum through two layers of filter paper in a Buchner funnel. Each filtrate is collected in a 500 mL glass filter flasks. Following the third extraction, the final volume of filtrate (composite of extracts 1, 2 and 3) is measured in a 500 mL graduated cylinder. Aliquots of each filtrate are placed in glass culture tubes for analysis by GC.

a. Calculations

The concentration of explosive (ppm) in the sample is read directly from the standard curve. The apparent concentration of explosive in the compost is calculated from the formula given below:

$$\text{Concentration (ppm)} = \text{ppm} \times \frac{\text{total extract volume}}{\text{g dry weight compost}}$$

b. Instrumentation

Gas chromatograph,- Hewlett-Packard 5880A with computer controller and integrator; auto injector and electron capture detector.

c. Parameters

Column - 2 ft x 2 mm I.D., 10% SE30 on 80/100 Supelcoport.

Temperature - injection port - 210°C
oven - 160-210°C
detector - 330°C
Temperature Programming - 10°C/min.
Carrier Gas - nitrogen at 30 cc/min.
Detector - electron capture
Injection volume - 2 μL
Retention Time - 0.36 min.

5. Liquid Scintillation Counting

The laboratory studies employed a ^{14}C tracer to follow the degradation or transformation of TNT and RDX in compost. Quantification of ^{14}C-activity was accomplished with a Beckman LS-7500 liquid scintillation counter. The counting window was set at 300 to 655. The lower limit of the window was set to avoid chemical fluorescence. The automatic quench control was employed to automatically adjust the window for quenched samples.

A standard quench correction curve was constructed from counting a series of sealed quenched standards and a sealed unquenched standard. All standards were counted twice until the 2 δ error reached 1%. All counts were corrected for background using a sealed reference background. An H number for each sample was determined using a ^{137}Cs external standard. The H number measures the shift in the Compton distribution due to quench and is therefore an accurate indicator of sample quench or counting efficiency. A plot of counting efficiency versus H number could not be accurately represented by a single linear or quadratic expression. However, the use of two quadratic equations for two sections of the curve did provide an accurate means to represent the curve mathematically. The point at which the two curves met was termed H_o and a counting efficiency was assigned at this point which was in agreement with both quadratic equations. The curve and its mathematic equivalent are presented in Figure 1.

A model TI-59C Texas Instrument calculator was programmed to correct counts per minute (CPM) for quench, background and dilution, concentration or subsampling and thus minimize computational errors.

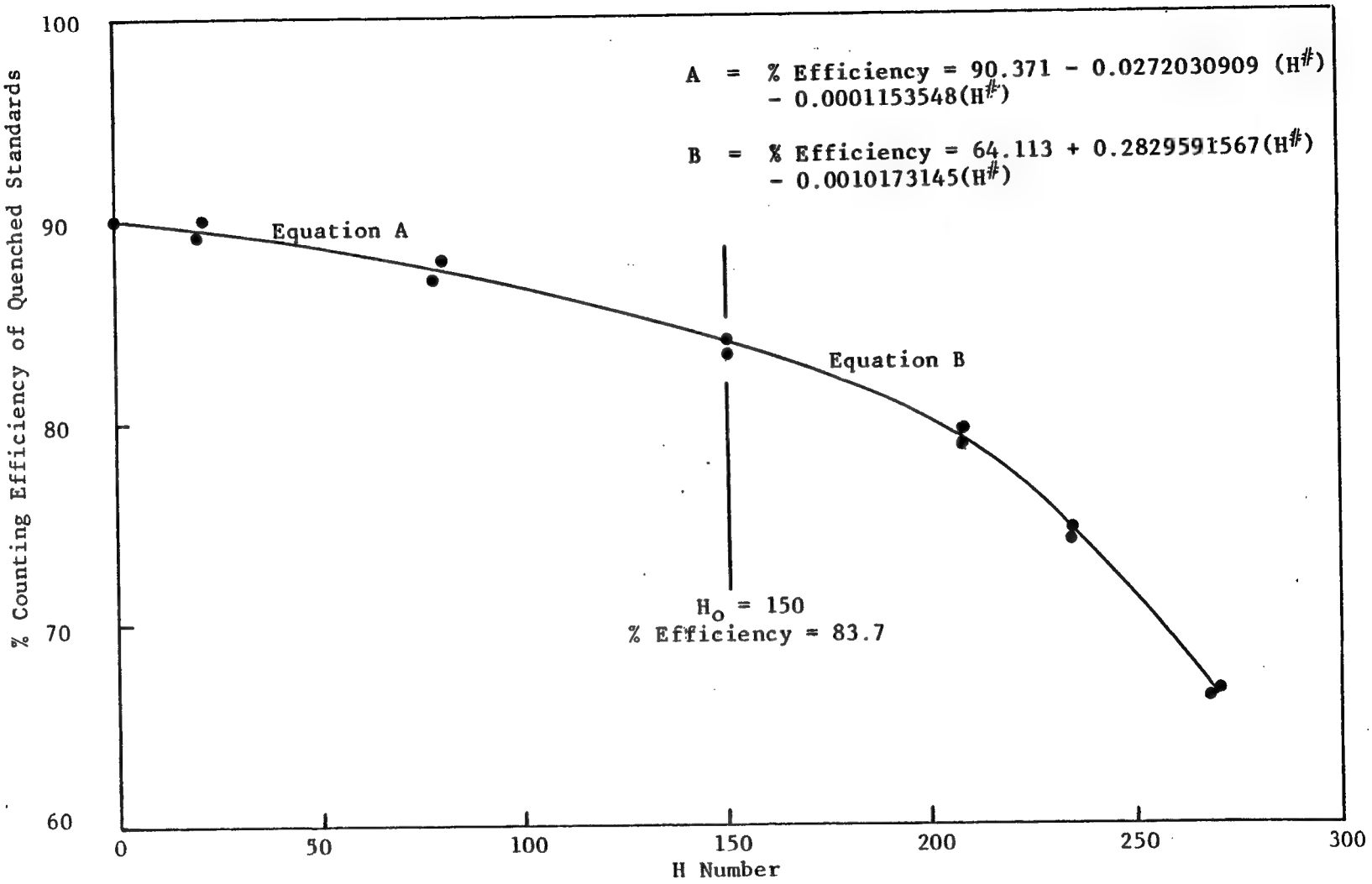


Figure 1. Quench Curve

6. ^{14}C-Product Identification and Quantification

Purity assays of ^{14}C-labeled explosives and quantification of ^{14}C-products produced during the composting of ^{14}C-explosives were accomplished using thin layer chromatography (TLC). One or more ^{14}C samples and appropriate non-labeled standards were spotted in separate spots on a single TLC plate. Chromatograph development was in a saturated atmosphere. The plates were then allowed to dry and X-ray film was placed on the plate for a set period of time. The developed X-ray film (autoradiograph) showed dark areas which corresponded with the ^{14}C-spots on the TLC plate. Spots containing ^{14}C were located and mapped on the TLC plate with the autoradiograph. The unlabeled standards and other fluorescent spots were located by exposing the TLC plates to shortwave (253.7 nm) ultraviolet light. Identification of the ^{14}C compounds was accomplished by comparing their R_f values with that of known standards. Quantification of the ^{14}C activity in each spot was accomplished by scraping the silica gel from the TLC plate directly into a scintillation vial, adding 10 mL of counting cocktail and counting the vial.

The following standards were used in TLC analysis:

For TNT analysis:	2,4,6-trinitrotoluene (TNT)
	2-amino-4,6-dinitrotoluene (2-amino-DNT)
	4-amino-2,6-dinitrotoluene (4-amino-DNT)
	2,6-diamino-4-nitrotoluene (2,6-diamino-NT)
	2,2',6,6'-tetranitro-4,4'-azoxytoluene (tetra)
For RDX analysis:	hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

7. ^{14}C-Detection Limit by TLC

An aliquot of the TNT solution at 1.97×10^7 DPM/mL was diluted with acetone and a 100 μL aliquot was counted. The sample contained 238.5 DPM. Aliquots of this stock solution were spotted on duplicate TLC plates. Spots contained approximately 30 DPM, 60 DPM, 90 DPM, 120 DPM, 190 DPM or 240 DPM. The plates were dried, film was placed on the plates and exposed for 8 days or for 14 days.

After 8 days of exposure, the 30 DPM spot was not visible on the autoradiograph. A faint spot was detected at 60 DPM and 90 DPM. Good spots were detected at 120, 180 and 240 DPM. The spots were scraped and counted.

At the end of 14 days of exposure, faint spots were detected at 30 and 60 DPM. Good spots were visible at 90, 120, 180 and 240 DPM. The plates were scraped and counted. Results are presented in Table 3.

Table 3. Detection Limit Data for ^{14}C on TLC Plates

Sample	8-day Exposure TLC DPM	14-day Exposure TLC DPM
30 DPM	-*	25
60 DPM	62	59
90 DPM	92	97
120 DPM	112	121
180 DPM	189	199
240 DPM	263	266

*No spot visible; not scraped

Based on this detection limit study, any fraction of the sample separated on the TLC plate containing as little as 30 DPM can be detected when the film is exposed for 14 days. Exposure of the film for 8 days allows for detection of fractions containing as little as 60 DPM.

8. Carbon and Residual - ^{14}C Determinations

Prior to analysis, all samples were freeze-dried and ground to a fine powder. Compost was ground in a ball mill. Activated carbon was crushed with a mortar and pestle. Subsamples (0.08 to 1.1 g) of the material to be analyzed were weighed into a ceramic combustion boat and covered with a 1:5 (by volume) mixture of cupric oxide and aluminum oxide. Each sample was combusted at 850°C for 30 minutes in a Hoskins electric furnace. The furnace was continuously flushed with O_2. For total carbon analysis, the combustion gases were scrubbed with 0.6 N NaOH to remove CO_2. A subsample of the NaOH trap was titrated to determine the quantity of carbon released during combustion. Carbosorb (Packard Instrument Co.) was used to absorb CO_2 released from the combustion of materials containing ^{14}C. The Carbosorb trap was mixed with an equal volume of Permafluor (Packard Instrument Co.) in a scintillation vial and the ^{14}C-activity was determined by liquid scintillation counting.

9. Moisture Determinations

The moisture content of compost and compost ingredients was determined by weighing 5 to 20 g of the material into preweighed beakers and drying the samples at 80°C for 24 hours. After drying, the samples were cooled in a desiccator before they were reweighed. The moisture was calculated as the weight loss during drying. The results are reported as percent moisture on a wet weight basis. A minimum of three subsamples were dried for each moisture determination and the average percent moisture value was used.

10. pH Determinations

The pH of individual composts was determined on a distilled water-compost slurry. Ten grams (wet wgt.) of compost were mixed with 30 mL of distilled water and allowed to sit for 45 minutes. The slurry was then stirred and the pH read immediately using standard calomel and glass electrodes with a pH meter. The average solid to liquid ratio of the slurry was 9:1 due to the moisture content of the compost.

11. Nitrogen Analysis

The total Kjeldahl nitrogen content of compost was determined using the Semimicro-Kjeldahl method described in Methods of Soil Analysis (1965). Prior to analysis, all samples were freeze-dried and ground to a fine powder.

12. Oxygen and Carbon Dioxide Determinations

The O_2 and CO_2 concentrations in the compost atmospheres were determined by gas chromatography analysis. A Varian 3700 GC was used with a 6 ft. CTR column (Alltech). Conditions used are given below:

Temperature -	injection port	- 200°C
	oven	- 65°C
	detector	- 260°C

Temperature Programming - isothermal

Carrier Gas - helium at 50 cc/min

Detector - thermal conductivity

Injection Volume - 80 μL

III. PRELIMINARY TNT LABORATORY COMPOST

A. Experimental Procedures

Three preliminary bench-scale composts were initiated in the laboratory to monitor breakdown of ^{14}C-TNT by composting and to establish solvent systems for separation of ^{14}C-containing compounds from each other and from other compost products.

The individual components of the compost were dried to determine the wet weight of each component required to prepare a specific (dry weight) compost mixture. Four alfalfa hay samples (10 g), four Purina Sweetena horsefeed samples (20 g) and four aliquots of the seed compost were used for the moisture determinations. The moisture contents of the hay, horsefeed and seed compost were 8.1%, 9.3% and 43.1%, respectively.

Using the predetermined moisture content of hay and horse feed, three composts were prepared as follows. Three 21 g (dry weight) hay samples were weighed into each of three one quart jars (0.95 L) and 67.0 mL of water were added to each jar. The jars were stoppered and allowed to stand for one hour to allow the hay to absorb the water. Horse feed (21 g dry weight) was added to each jar, the contents were mixed and the jars stoppered and allowed to equilibrate overnight.

Samples of Lakeland soil were weighed (14.5 g) into each of three beakers. Approximately 1 µCi of ^{14}C-TNT in acetone (0.22 mL) was added to the sand in each of two beakers. Production grade TNT dissolved in acetone (1.76 mL containing 0.4967 g TNT) was also added to the sand in each of the three beakers. The beakers were covered and placed in a hood in the dark to evaporate the acetone.

On day two, 3 g (dry weight) of the seed compost were added to each of the compost jars containing hay and horsefeed. The TNT contaminated soil (dry) from one beaker was scraped into one compost jar with a rubber policeman. The second and third beakers were also scraped into individual compost jars. An additional 1.7 mL of water were added to each jar to bring the total water content of the compost flasks to 75.0 mL (56% moisture content). All components (hay, horsefeed, seed compost, soil and water) were well mixed.

A ring of plastic tubing with holes drilled at 1/4 inch intervals was located beneath the compost in each jar. The ring was connected to a glass tube extending through the stopper to provide aeration for the experimental composts. A thermocouple for monitoring of compost temperature was placed in the center of each compost. As shown in Figures 2 and 3, each jar was securely stoppered, all tubing attached to the proper trap or vacuum system and the vacuum was applied to pull air through the compost materials. Air was drawn successively through a NaOH and a H_2O trap to remove CO_2 and humidify the air before entering the compost. Gases exiting the compost were passed through H_2SO_4 (36N), NaOH (5N) and activated carbon traps to retain volatile ^{14}C materials resulting from the breakdown of ^{14}C-TNT.

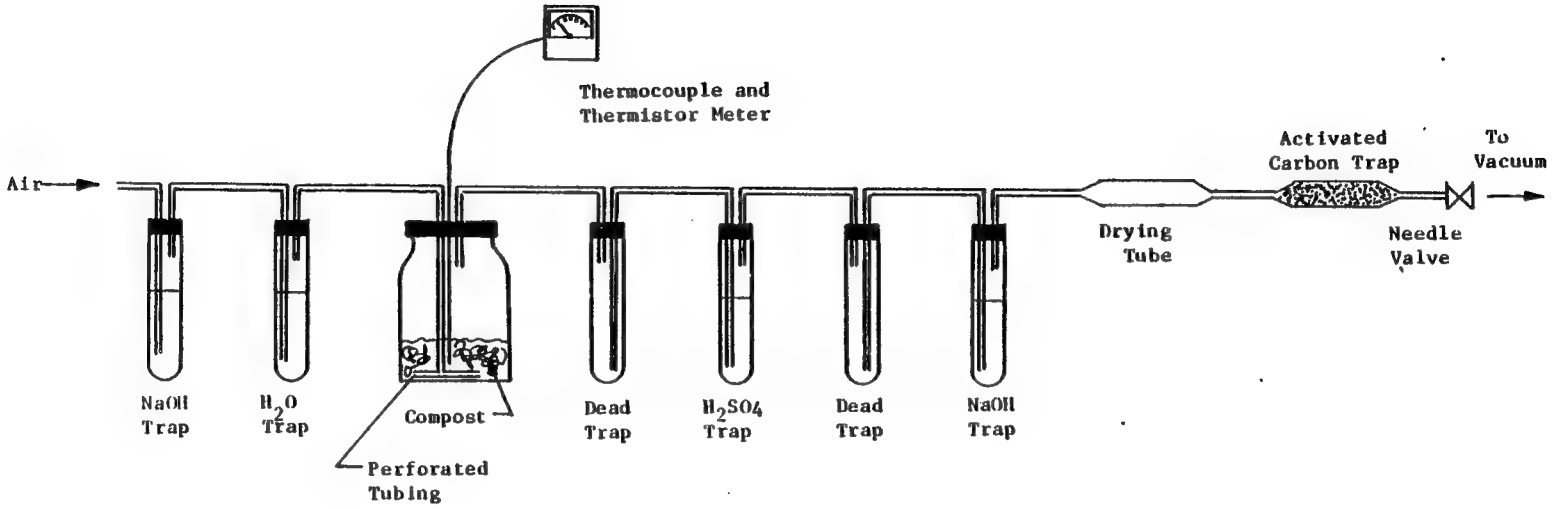


Figure 2. Schematic of ^{14}C-Bench-Scale Composting Apparatus

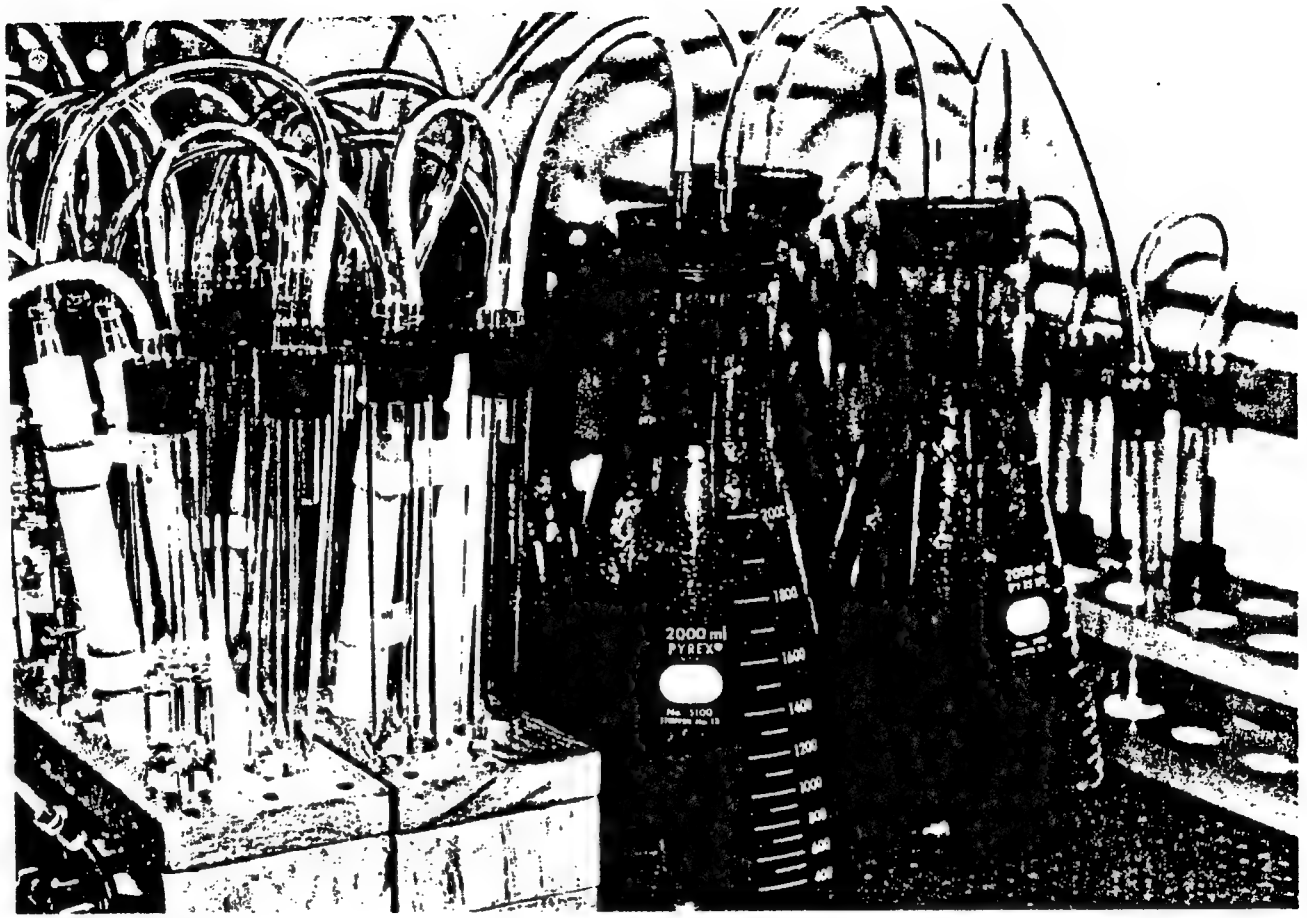


Figure 3. Picture of a Bench Scale Composting Apparatus

An unlabeled compost (control - contained TNT but no ^{14}C-TNT) was set-up in the same manner. The aeration system for the unlabeled compost was simplified as illustrated in Figure 4.

B. Results of Preliminary TNT Composting Study

1. Routine Monitoring of Composts

The temperature of each compost and the air temperature of the incubator were recorded once daily. The results are compiled in Table 4. Generally there was little difference between the compost and incubator temperatures.

Samples of the compost atmosphere were removed via the cannula (Figure 4) from each control compost and analyzed by gas chromatography on a weekly basis. Oxygen levels in the compost atmosphere were determined to lie between 4 and 7% at all times sampled during the three week incubation.

The NaOH traps were changed every seven days, or more often if the traps neared saturation with CO_2. A one mL aliquot of the trap was counted for 60 minutes to determine ^{14}C activity. The results in Table 5 show that ^{14}C recovery from the NaOH traps was very low. Average recovery over the three-week composting period totaled 0.08% of the ^{14}C originally added to the compost.

The H_2SO_4 traps were sampled after 6, 14 and 21 days of composting. One mL of the trap was removed at each sampling and counted for 60 minutes. No significant ^{14}C-activity was detected in the samples. The results are given in Table 6.

2. Extraction of Three-Week Preliminary Laboratory ^{14}C-TNT Composts

The two composts containing ^{14}C-TNT were extracted three times with 400 mL warm (37°C) acetone. The 400 mL aliquot of acetone was added to the compost in the jar which was placed in a water bath at 37°C. The jar was agitated at 5 minute intervals and removed from the water bath at the end of 15 minutes. The extract was vacuum filtered (Whatman #2 filter paper). The procedure was repeated two additional times and the extracts were combined and brought to a final volume of 1125 mL. Aliquots (1 mL and 200 μL) of the 1125 mL combined extracts were counted. In Sample A, 50.8% of the ^{14}C was recovered and 62.3% of the ^{14}C was recovered in Sample B.

3. Analysis of Preliminary Compost Extracts

Compost extracts were stored in the dark to prevent photoreduction of TNT. However, after several days storage in the dark at room temperature, all

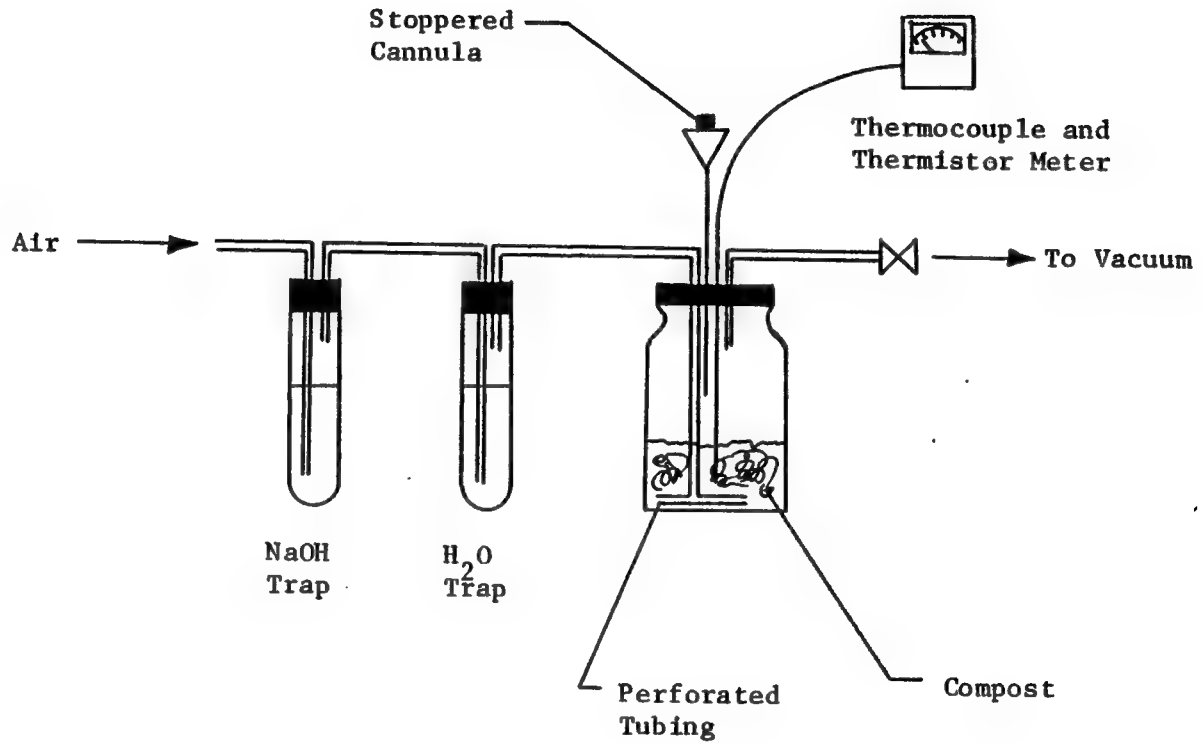


Figure 4. Schematic of Unlabeled (Control) Bench-Scale Composting Apparatus

Table 4. Daily Temperature Readings for the Preliminary Composts

	^{14}C-Labeled TNT Compost A	^{14}C-Labeled TNT Compost B	Unlabeled TNT Compost (control)	Incubator
9/13/81	54.0	53.0	53.0	52
9/14/81	54.0	53.0	53.0	52
9/15/81	52.0	53.0	53.0	54
9/16/81	53.0	53.0	53.0	54
9/17/81	53.0	52.0	53.0	53
9/18/81	53.5	53.0	53.0	54
9/21/81	53.0	53.0	53.0	54
9/22/81	54.0	54.0	54.0	55
9/23/81	53.0	53.0	53.0	55
9/24/81	53.0	53.0	53.0	55
9/25/81	54.0	54.0	54.0	55
9/28/81	52.0	51.5	52.0	55
9/29/81	52.0	52.0	52.0	54
9/30/81	52.5	52.5	52.5	54
10/01/81	53.5	53.5	53.5	57
10/02/81	53.0	53.0	53.0	54

Table 5. ^{14}C-Activity in NaOH Traps from the Preliminary Composts

Sample	Total DPM	% ^{14}C Recovered
6 day Compost A	334	0.02
Compost B	272	0.01
14 day Compost A	316	0.02
Compost B	768	0.04
20 day Compost A	304	0.01
Compost B*	927	0.04
21 day Compost A	185	0.01
Compost B	106	0.01

*Traps saturated.

Total ^{14}C recovery as CO_2 A - 0.06%
B - 0.10%

Table 6. ^{14}C Recovery in H_2SO_4 Traps from the Preliminary Composts

Sample	Total DPM	% ^{14}C Recovered
6 day Compost A	0	0.00
Compost B	0	0.00
14 day Compost A	0	0.00
Compost B	0	0.00
21 day Compost A	20	0.00
Compost B	67	0.00

extracts turned a dark red color indicating that TNT was reduced. The three-week acetone compost extracts were evaporated to dryness with a rotary vacuum evaporator. The dried extracts were redissolved in benzene followed by sequential acetone and methanol washings. The benzene contained 91.6% of the radioactivity contained in the original acetone extract. The combined recovery of radioactivity in acetone and methanol was 2.6% of the total radioactivity in the original acetone extract. The extract dissolved in benzene was analyzed by TLC using eight solvent systems (see Table 7). Autoradiographs indicated that solvent systems #1, 2 and 4 gave the best separation of the extract components. The three radioactive spots present on each chromatograph were tentatively identified as TNT and the 2-amino and 4-amino reduction products of TNT. No further analysis of the extracts was attempted because of the obvious problem with TNT reduction in the extracts.

4. Conclusions Based on Preliminary TNT Compost Experiment

The initiation and incubation of the preliminary composts identified a number of minor problems which were corrected with only slight modification in the proposed set-up. The major problem identified during the preliminary compost period was that acetone extracts of the TNT compost were not stable even when all possible precautions were taken, i.e. removal from light, storage at low temperatures and limited storage times. An extraction procedure using benzene:methanol was developed for use in the subsequent laboratory and greenhouse compost experiments to avoid the problem of instability.

Three of eight solvent systems investigated for TLC separation of TNT extract components yielded good separation of TNT from the amino products formed by reduction of TNT. Solvent system #8 was found to separate TNT from 2,2',6',6'-tetranitro-4,4'-azoxytoluene. By combining solvent systems #8 and #2 in a two dimensional TLC development, TNT and all its transformation products for which standards were available could be separated.

Temperatures in ^{14}C-labeled and unlabeled TNT compost jars were approximately the same, ranging from 51.5 to 54°C. Based on the oxygen analysis of the compost atmosphere, the compost was aerobic at all times. Little of the ^{14}C introduced into the compost as TNT was recovered in the sodium hydroxide traps, indicating that ring cleavage probably did not occur. No significant ^{14}C-activity was found in the acid traps or in the carbon traps, indicating that volatile amines and volatile aromatic compounds were not produced in detectable quantities during laboratory composting. Based on recovery of ^{14}C in three-week compost extracts, 40-50% of the ^{14}C introduced into the compost was no longer solvent extractable. TLC analysis of the compost extract gave three radioactive spots. The majority of the radioactivity on each plate (88.3 and 89.2%) was contained in a spot with an R_f corresponding to the TNT standard. A small percentage of the ^{14}C (3 to 8%) was tentatively identified as the 2-amino and 4-amino-DNT reduction products. Polar products at the origin of the plate accounted for 3 to 4% of the ^{14}C-activity.

Table 7. TLC Solvent Systems Evaluated for TNT Analysis*

1. Toluene:benzene:hexanes (10:10:5)
2. Benzene:hexanes:pentane:acetone (50:40:10:3)
3. Hexanes:acetone (3:2)
4. Chloroform
5. Chloroform:methanol:acetic acid (8:20:1)
6. Chloroform:ethyl acetate (3:2)
7. Benzene:ethyl acetate:acetic acid (15:10:1)
8. Petroleum ether:ethyl acetate:hexanes (160:80:25)

*All systems on a volume to volume basis.

IV. LABORATORY COMPOSTING OF TNT AND RDX

A. Compost Set-Up

Composts containing approximately 1% TNT or RDX were set-up essentially as were the composts in the preliminary study. Nine composts for each explosive were prepared to be sampled in triplicate at 0, 3 and 6 weeks of composting. Each of these composts was dosed with one ^{14}C-labeled explosive (TNT or RDX) to monitor the degradation of TNT and RDX. Five additional control composts for each explosive were set up to monitor the pH, moisture, O_2, carbon and nitrogen content of the composts. One of these control composts was sacrificed (the entire compost sample was extracted) at time zero. Two of the control composts for each explosive were sacrificed after 3 and 6 weeks of composting. A summary of the laboratory composting system is presented in Table 8.

1. Soil Spikes

TNT contaminated soil was prepared by adding 2.1 mL of acetone containing 0.4969 g of production grade TNT and 0.22 mL of acetone containing 1.06 µCi of ^{14}C-labeled TNT to 10 g of air dried soil in a 50 mL beaker. RDX soil spikes were made by adding 13.1 mL of acetone containing 0.6144 g of production grade RDX and 0.85 mL of acetone containing 0.72 µCi of ^{14}C-labeled RDX to 10 g of soil. Control soils were spiked with the same quantities of production grade explosive but no ^{14}C-labeled material was added. One soil sample was prepared for each compost. The beakers containing the dosed soil were wrapped in aluminum foil and allowed to dry overnight in the dark, in a hood at room temperature.

2. Laboratory Composts

The moisture contents of the hay, horsefeed and seed compost used were determined by drying triplicate samples for 24 hours at 80°C. The weights of these materials and the water added to the compost were adjusted for the moisture levels of the starting material. Hay (18.5 g dry weight) was weighed into quart size glass jars and 56.4 mL of distilled water were added to each jar. Horse feed (18.5 dry weight) was added to each jar; the contents were well mixed and the jars stoppered. Seed compost (3 g dry weight) was added to each of the jars containing hay and horsefeed. The soil containing TNT (or RDX) from one beaker was scraped into one compost jar with a rubber policeman. The beaker was rinsed twice with approximately 1 mL acetone. The acetone rinses were added to the compost jars. Each beaker was treated in the same manner. An additional 11 mL of water were added to each jar to bring the total water content of the compost jars to 75 mL (60% moisture content). All components (hay, horsefeed, seed compost, soil, water) were thoroughly mixed with a glass stirring rod.

Table 8. Summary of Laboratory Compost Systems

1) Compost - 50 g (dry weight) hay and horsefeed compost; initial moisture content adjusted to 60% (wet weight basis).

   a. dosed with TNT (1%), included uniformly ring labeled ^{14}C-TNT at a specific activity of 2.13 µCi/g or

   b. dosed with RDX (1%), included uniformly labeled ^{14}C-RDX at a specific activity of 1.17 µCi/g or

   c. control composts contained 1% TNT or RDX, no ^{14}C-labeled explosives added.

2) Composting conditions:

   a. incubated at 55°C

   b. continuously aerated with humidified and warmed CO_2 free air

   c. off-gases scrubbed through H_2SO_4, NaOH and activated carbon traps

3) Sampling procedures:

   a. three replicate ^{14}C composts sacrificed at 0, 3 and 6 weeks of composting to monitor TNT or RDX disappearance

   b. two replicate control composts sacrificed at 3 and 6 weeks of composting to monitor pH, moisture level, carbon and nitrogen contents

   c. H_2SO_4 and NaOH traps changed as needed to prevent trap saturation

   d. temperature monitored daily

   e. O_2 and CO_2 levels in control composts monitored weekly

4) Analysis:

   a. RDX, TNT, TNT transformation products quantified by TLC and liquid scintillation counting (LSC) of compost extracts

   b. residual ^{14}C in the compost determined by combustion followed by LSC

   c. H_2SO_4 and NaOH traps assayed for ^{14}C by LSC

   d. ^{14}C retained in activated carbon quantified by combustion and LSC

   e. O_2 and CO_2 levels determined by GC.

The beakers that contained the dosed soils were washed with an additional 3 mL of acetone to remove residual ^{14}C. An average of 4972 DPM of TNT and an average of 8939 DPM of RDX remained in the acetone wash of the beakers. The quantities of explosives and ^{14}C-labeled material added to each flask are summarized in Table 9.

The composts to be sacrificed at time zero were randomly selected. The ^{14}C composts were extracted by appropriate methods immediately. The control composts were freeze dried and stored until carbon and nitrogen analyses were performed.

The remaining composts were connected to an appropriate aeration system (Figures 3 and 4) as described in the Preliminary TNT Laboratory Compost section and incubated at 55°C. The aeration system removed CO_2 from the air, saturated it with water, then drew the air through the compost. Air entering this system was from inside an incubator (55°C) and therefore did not cool the compost. Humidification of the air pulled through the compost maintained an acceptable moisture content in the compost. Off-gases from the compost were scrubbed through concentrated H_2SO_4 to trap volatile amines (possible metabolites from TNT degradation), through 5 N NaOH to trap $^{14}CO_2$, through a drying tube ($CaSO_4$) to remove excess moisture and through activated carbon to remove volatile aromatics.

B. Results of Laboratory Compost Studies

1. Routine Monitoring of Composts

The procedures used for monitoring the laboratory composts are outlined in Figure 5 and described in detail in the following paragraphs. The temperature of each experimental compost was monitored and recorded daily. In general, the compost temperatures ranged from 51 to 55°C as did the temperature of the incubator. Compost temperatures during the second three-week incubation were higher with some readings as high as 59°C, a reading at least 2°C higher than the temperature in the incubator. The daily temperature readings are compiled in Appendix D.

$^{14}CO_2$ resulting from TNT and RDX breakdown in the compost was trapped by bubbling all off-gases through NaOH. The traps were changed frequently (every 5 days or less). The cumulative evolution of $^{14}CO_2$ during RDX composting is illustrated in Figure 6. Each point on the curve represents an average of three replications. The recovery of ^{14}C activity as $^{14}CO_2$ from the TNT composts was very low. Recoveries ranged from 0.2 to 0.6% of the ^{14}C added to the compost. Average recoveries from the 3 and 6 week composts were 0.2 and 0.5%, respectively.

Table 9. Quantity of RDX and TNT Added to Individual Laboratory Composts

Compound	Specific Activity (μCi/mg)	μ Curies Added	Explosive Added (mg)	Concentration of Explosive in Compost*
^{14}C-TNT	25.59	1.0603	0.0414	1.07%
Production TNT	-	-	0.4959	
^{14}C-RDX	312.50	0.7200	0.0023	1.45%
Production RDX	-	-	0.6110	

*Corrected for explosive not transferred from beaker into the compost.

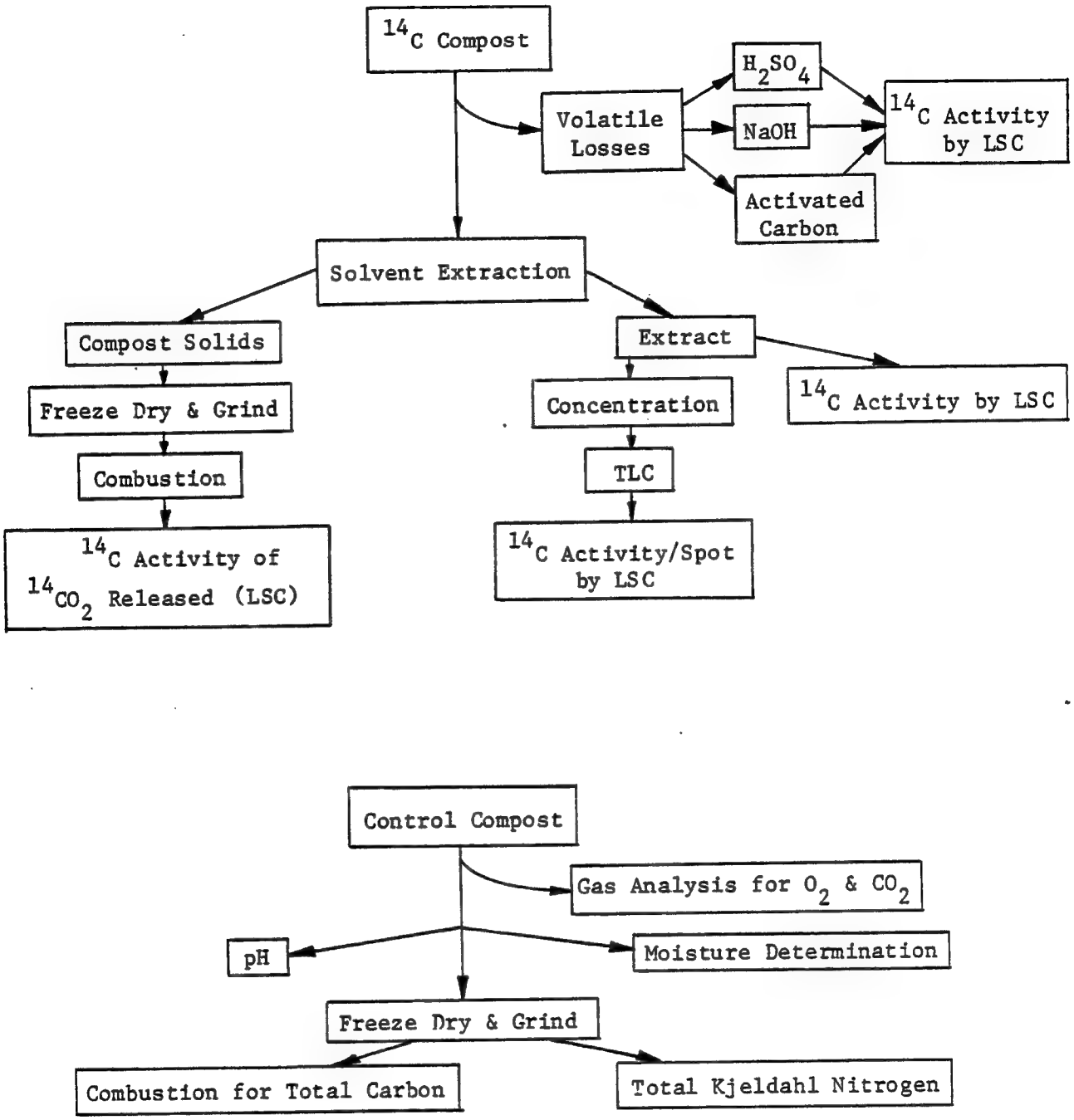


Figure 5. Schematic for Monitoring Laboratory Composts

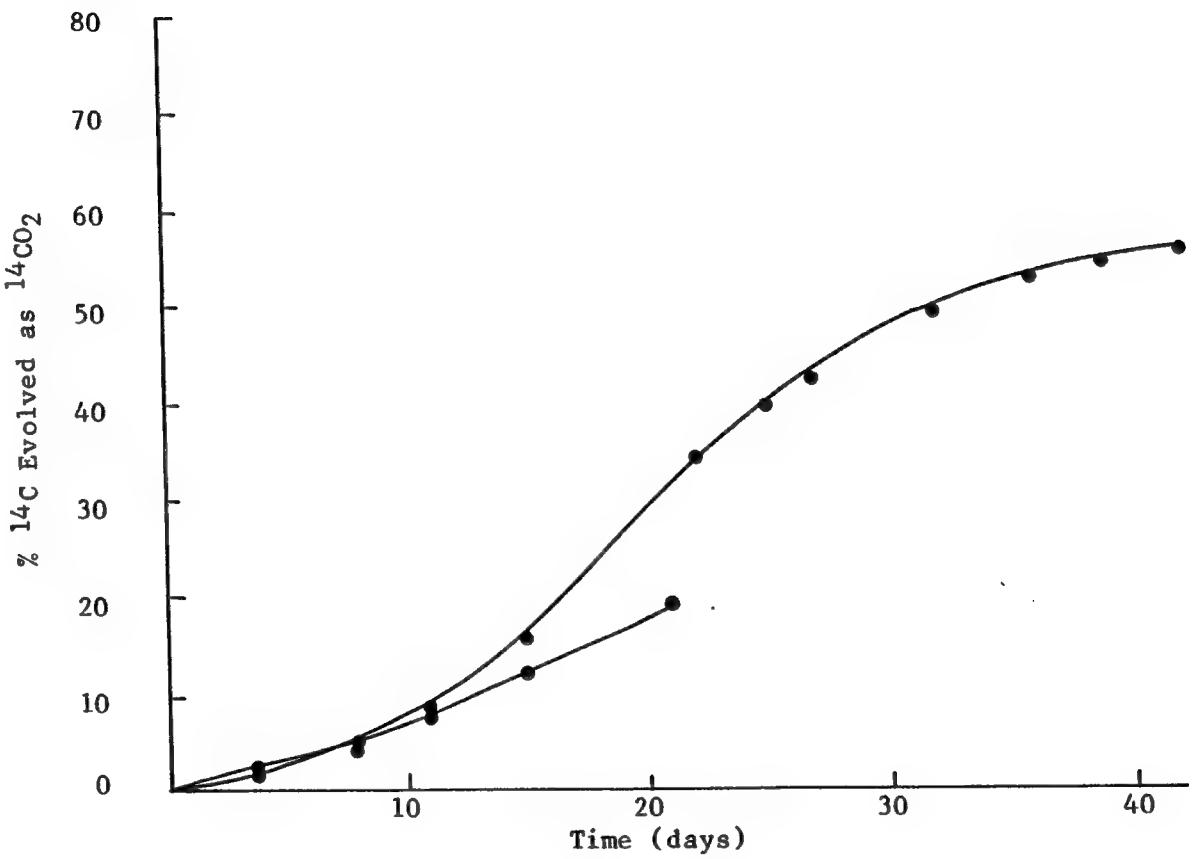


Figure 6. Cumulative Percent ^{14}C Recovered as $^{14}CO_2$ from ^{14}C-labeled RDX in Compost

Recovery of ^{14}C from the H_2SO_4 traps was low for both TNT and RDX composts. Average cumulative recoveries after 3 and 6 weeks of composting are given in Table 10.

Table 10. Average Cumulative Recoveries of ^{14}C-activity from H_2SO_4 Traps

Explosive	Composting (week)	Cumulative ^{14}C Recovery (%)
TNT	3	0.0
	6	0.2
RDX	3	0.3
	6	0.7

The activated carbon traps were sampled at the completion of the experiment. The carbon was thoroughly mixed and a subsample was removed and crushed to a fine powder with a mortar and pestle. Two subsamples of the crushed carbon were combusted to release the ^{14}C for liquid scintillation counting. The combustion method was described in Section IIC-8. The recovery of ^{14}C for both RDX and TNT from the activated carbon trap was essentially zero (background level).

The jars containing the control composts were fitted with a stopper containing a septum through which samples of the compost atmosphere could be withdrawn. Once a week duplicate samples were taken from each control compost for GC analysis to determine the oxygen (O_2) and carbon dioxide (CO_2) levels in the compost. The GC conditions and column used are described in Section IIC-12. Both the O_2 and CO_2 levels were highly variable among replicate samples (See Table 11). The O_2 levels and CO_2 were inversely related. The O_2 content was sufficiently high in all samples to avoid anaerobic conditions.

2. Analysis of Control Compost

For week 0, one TNT and one RDX control compost were sacrificed for analysis. At weeks 3 and 6, analyses were performed on duplicate control composts of each exlosive. The composts to be sacrificed were randomly selected. Two subsamples of each compost were removed. One was dried at 80°C for 24 hours to determine the moisture content. The second subsample was combined with distilled water (approximately a 1:9 (w/v) solid to water combination), allowed to stand for 45 minutes, and then the pH of this slurry was measured using standard calomel and glass electrodes. The remaining compost was freeze-dried and then ground to a fine powder in a ball mill Two subsamples of the powdered compost were combusted to determine total carbon, and one or two subsamples were analyzed for Kjeldahl nitrogen (see Section IIC-11 for methods). Results are summarized in Table 12.

Table 11. Oxygen and Carbon Dioxide Levels in Control Compost Atmospheres

	TNT				RDX			
	O_2 (%)		CO_2 (%)		O_2 (%)		CO_2 (%)	
Composting (days)	$\bar{X}$*	S	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
8	15.9	5.3	11.4	7.8	16.0	5.8	8.8	5.7
15	21.8	1.9	4.0	2.6	19.7	5.3	6.0	6.5
22	17.2	11.1	10.4	14.7	16.1	14.1	14.0	14.6
27	18.4	93.9	4.2	6.5	21.9	1.1	4.2	2.7
40	8.9	10.0	12.5	12.9	17.3	1.5	4.2	3.3

* $\bar{X}$ = Arithmetic mean

S = Standard deviation

Table 12. Analysis of Control Composts for Laboratory Study

Sample		Length of Composting (week)	pH	Percent Moisture	Total Carbon	Total Nitrogen
TNT	1	0	5.9	60.0	32.8	2.1
	2	3	8.1	58.8	29.8	2.0
	3	3	6.0	59.7	33.7	1.9
	4	6	8.0	61.9	26.8	2.0
	5	6	4.7	56.3	32.9	1.6
RDX	1	0	5.9	60.0	31.0	2.1
	2	3	8.3	66.3	25.5	1.9
	3	3	4.8	53.1	32.3	1.7
	4	6	8.5	64.5	22.8	2.0
	5	6	8.8	70.1	22.9	1.8

3. Analysis of ^{14}C-labeled Composts

At 0, 3 and 6 weeks of composting, three jars containing ^{14}C-labeled TNT and 3 jars containing ^{14}C-labeled RDX compost were selected at random to be sacrificed. The entire contents of each jar were extracted by the method described in Section II for extraction of TNT or RDX from compost. An aliquot of the extract (0.5 mL or 1 mL) was counted for ^{14}C-activity by liquid scintillation counting to determine what percentage of the total ^{14}C-radioactivity added to the compost was recovered in the extract. The remainder of the extract was stored in sealed glass containers at room temperature in the dark until rotary vacuum evaporation was carried out in preparation for TLC analysis. The results are summarized in Tables 13 and 14. RDX and TNT composts demonstrated a dramatic decrease in extractable ^{14}C-activity as the length of composting increased.

Following extraction, the compost solids were freeze-dried, then weighed and powdered by grinding in a ball mill for two or more hours. Duplicate subsamples were combusted to determine the residual ^{14}C-activity in the compost. The results are given in Tables 13 and 14. The total residue activity was corrected for weight loss during composting.

The compost extracts were concentrated by rotary vacuum evaporation to dryness. The dried extract was washed out of the drying flask with 8 to 12 mL of solvent (benzene for TNT, acetone for RDX). The solvent containing explosive was then reduced in volume to approximately 0.5 mL by blowing N_2 across the sample. A suitable aliquot (5-20 μL) of this concentrated extract was analyzed by TLC. The TLC procedures are described in Section IIC-6. The TNT analyses performed use two-dimensional TLC plates. The solvent systems used were petroleum ether:ethyl acetate:hexanes in ratios of 160:80:25 (solvent system #8) and benzene:hexanes:pentane:acetone combined in ratios of 50:40:10:3 (solvent system #2). The separation of TNT and its transformation products in this system is illustrated in Figure 8. Solvent system #8 travels from left to right across the plate separating the mono- and diamino derivatives of TNT. Solvent system #2 moves from the bottom to the top of the plate separating TNT from the tetranitroazoxy derivatives. The results for each individual extract are given in Table 15. Only TNT was detected at time zero. After three weeks of composting 45 to 49% of the ^{14}C initially added to the compost was recovered as TNT and a small percentage of ^{14}C was found at the origin. After six weeks of composting, the TNT levels were further reduced (0 to 37% of ^{14}C recovered as TNT). Small quantities of the TNT transformation products were found and between 0.9 and 2.0% of the total ^{14}C activity did not move from the origin on the TLC. In one replicate, when no TNT was recovered, two new unidentified radioactive spots were seen on the TLC. The ^{14}-activity of these spots was low, with less than 0.5% of the total activity found in either spot.

Table 13. Summary of ^{14}C Recovered from ^{14}C-TNT Laboratory Composts

Length of Composting	% Recovery of ^{14}C					
	$^{14}CO_2$	H_2SO_4 Trap	Carbon Trap	Solvent Extract	Residual-^{14}C	Total
0 weeks	0.0	0.0	0.0	93.5	1.7	95.2
3 weeks	0.2	0.0	0.0	47.8	37.8	85.8
6 weeks	0.5	0.0	0.0	19.3	66.5	86.3

[1]Total ^{14}C-activity in compost extraction present as ^{14}C-TNT and other ^{14}C-compounds.

Table 14. Summary of ^{14}C Recovered from ^{14}C-RDX Laboratory Composts

Length of Composting	% Recovery of ^{14}C					
	$^{14}CO_2$	H_2SO_4 Trap	Carbon Trap	Solvent Extract[1]	Residual-^{14}C	Total
0 weeks	0.0	0.0	0.0	112.3	6.1	118.4
3 weeks	19.6	0.3	0.0	68.9	13.5	102.3
6 weeks	55.8	0.7	0.0	21.6	16.1	94.2

[1]Total ^{14}C-activity in compost extract present as ^{14}C-RDX.

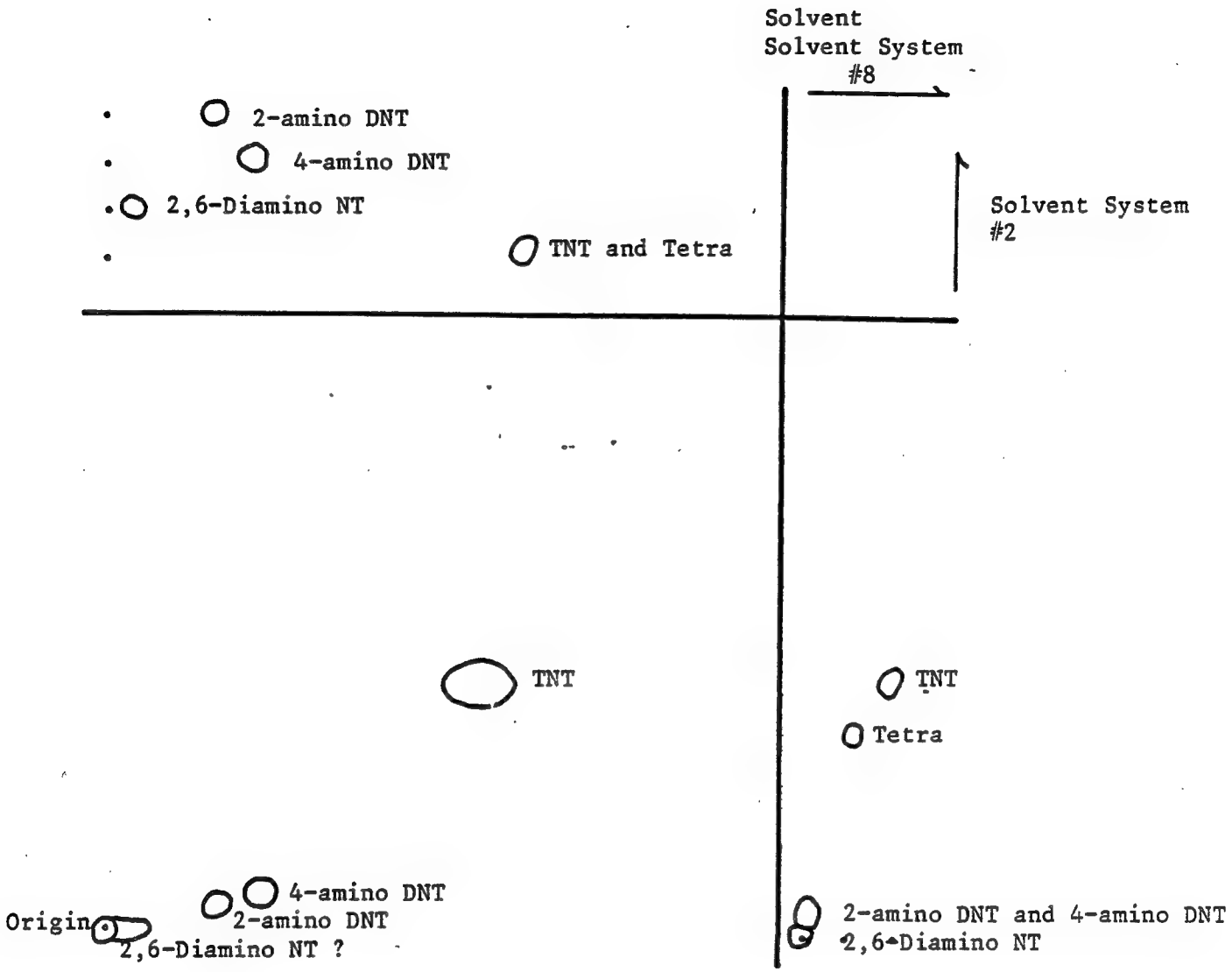


Figure 7. Separation of ^{14}C-TNT by TLC

Table 15. ^{14}C Recovery from TNT Laboratory Compost Extracts

Length of Composting	Replicate	TNT	% of Total ^{14}C				
			A*	B	C	D	E
0 weeks	A	89.8					
	B	88.8					
	C	101.8					
3 weeks	A	44.5				1.5	
	B	48.9				1.0	
	C	46.5				0.9	
6 weeks	A	N.D.			0.1	1.0	0.6**
	B	37.0			1.1	2.0	
	C	12.9	0.6	1.1	0.8	0.9	

*A - 2-amino-2,4-dinitrotoluene

B - 4-amino-2,6-dinitrotoluene

C - this was not a discrete spot on any chromatograph but an area that would contain 2,6-diamino-4-nitrotoluene if it was present

D - origin

E - other unidentified ^{14}C-compounds

**Present in two spots

N.D. - Not Detected

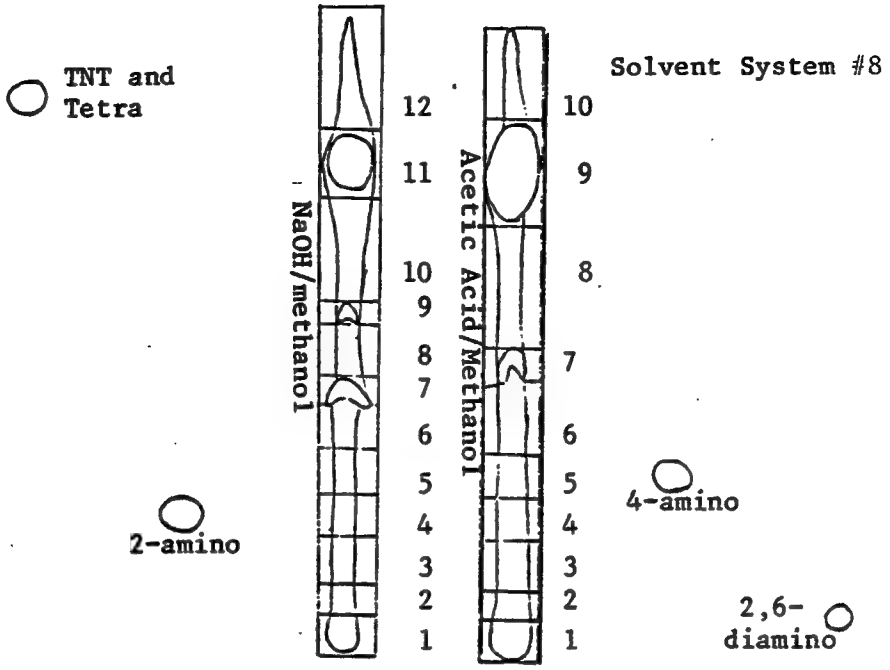
A second set of extractions was performed to ensure that the amino transformation products of TNT would be detected if they existed. The "C" replicate of the 6-week old compost was chosen as a test sample because it was the only compost where detectable quantities of the 2-amino, 4-amino, and 2,6-diamino derivatives of TNT were tentatively identified in the benzene extract. Aliquots of the compost, after being freeze-dried and ground were extracted with acidified methanol (pH 4.5) or with basic methanol (pH 11.5-12) to extract any amino derivatives in the TNT compost which had not been removed by the benzene extraction. One gram of dry powdered compost ("C") was extracted with 5 mL of acidic or basic extracting solution, centrifuged and an aliquot of the extract counted for radioactivity. Results of these extractions are given in Table 16.

Table 16. ^{14}C in Acid and Basic Methanol Extracts from ^{14}C TNT Laboratory Composts

Extract	Vol. Counted	DPM (total)	% ^{14}C
Acid	0.5 mL	131699	5.6
	1.0	133023	5.7
Basic	0.5	120520	5.1
	1.0	122369	5.2

The remainder of each extract was concentrated by evaporation with nitrogen and analyzed by TLC using solvent system #8. The ^{14}C-activity on the TLC plate was relatively low. Therefore, spots were visualized with UV light and the regions between spots were divided in segments. Each spot and segment was scraped from the plate and assayed for ^{14}C-activity. As shown in Figure 8, three spots were visualized with UV in the acid extract and two spots in the basic extract. Fraction 11 (acid) and 9 (base) corresponding to TNT and tetra and fraction 1 from each extract (origin) were the only areas giving counts which were significantly above background. No 2,6-diaminonitrotoluene, 2-amino or 4-amino-DNT were found in these extracts.

The solvent system used for TLC analysis of the ^{14}C-RDX extracts was cyclohexanone. Chromatographs for individual compost extracts showed that the only ^{14}C-labeled compound present in the extracts from sampling times zero, 3 weeks, and 6 weeks was ^{14}C-RDX. The three-week compost extract contained 68.9% of the ^{14}C-added to the compost whereas composting for six weeks resulted in an average recovery of 21.6% of the ^{14}C-RDX in the compost extract. Evolution of $^{14}CO_2$ during six weeks of composting was significant, and ^{14}C-recoveries as $^{14}CO_2$ greater than 67% in individual compost replicates were observed.



TLC ID	Acid Extract DPM*	Acid Extract Compound	Basic Extract DPM*	Basic Extract Compound
1	27.0	origin	42.3	origin
2	6.3	2,6-diamino	4.4	2,6-diamino
3	5.0		3.6	
4	3.6	2-amino	5.0	2-amino
5	5.2	4-amino	1.5	4-amino
6	2.4		2.7	
7	3.2		1.7	
8	2.1		3.7	
9	2.9		33.1	
10	11.3		2.1	TNT & Tetra
11	26.1	TNT & Tetra**		
12	4.9			

* DPM corrected for quench and background

** 2,2',6,6'-tetranitro-4,4'-azoxytoluene

Figure 8. TLC Analysis of Acidic and Basic Extracts of ^{14}C-TNT Laboratory Compost

4. Statistical Analyses of Data from Laboratory Composts

Data obtained from composting of RDX and TNT in the laboratory were statistically analyzed using a one-way analysis of variance. This test compares the variance attributed to random variation in the total population being observed to the average variance resulting from treatments being applied to the population. The ratio of the treatment variance to the sample population variance (error or replication variance) is termed the F value. If this ratio equals one, the treatment variance equals the population variance; thus the treatment has no affect on the population. As the F ratio increases above the value of one, the probability that the treatment has altered the population increases. Probabilities associated with F ratios for varying sample sizes are commonly available in most statistical tables.

In the present situation, the treatment is length of time composted. The ANOVA tests to see if the parameters measured (such as TNT concentration) have been significantly changed by 3 or 6 weeks of composting. The ANOVA tables (Tables 17 and 18) present the degrees of freedom and the sums of squares. These values are used to calculate the mean squares which are equivalent to variances. The probability for the F ratio is the probability that treatment differences are not real.

A requirement for using ANOVA is that the data possess homogeneity of variance. A portion of the data for both RDX and TNT lacked homogeneity of variance at the 5% level of probability according the Cochran's test (Chemical Rubber Company Handbook, 1968). Several transformations were used to equalize variances. However, variances varied independently of means and no transformation corrected the lack of homogeneity for all data. The square root transformation (x + 1/2) corrected the non-homogeneity of variance for all RDX data but not for TNT data. Analysis of variance was performed on both RDX and TNT results using non-transformed data, as well as using data with the square root transformation. An additional test to examine the equality of means when the variances are heterogeneous was used to analyze TNT data (Sokal and Rohlf, 1969). With one exception (^{14}C-recovered from the H_2SO_4 during RDX composting) F-ratios were highly significant with predicted probabilities of less than 0.01. The probabilities predicted from the F-ratios of these analyses were of the same order of magnitude regardless of the transformation or type of test. These results indicate that the lack of homogeneity of variance did not appreciably alter the results of the analysis of variance. Therefore, all analyses were performed on non-transformed data. A one-way analysis of variance was used to test each parameter (i.e. $^{14}CO_2$, solvent extract, etc.) separately for TNT and RDX. The recovery of TNT in the solvent extract was also tested. The ANOVA's for TNT and RDX are shown in Tables 17 and 18, respectively. When significant differences were indicated by the analysis of variance, the Student-Newman-Kuel Multiple Range Test was used to separate means. All testing was done at the 5% level of significance.

Results of the Student-Newman-Kuel Multiple Range Test showed that the $^{14}CO_2$ recovered from the TNT laboratory composts at time zero was not significantly different from that recovered by 3 weeks; however, $^{14}CO_2$

Table 17. Analysis of Variance Tables for the TNT Laboratory Composts

Parameter	Source of Error	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Probability
$^{14}CO_2$	Time	2	0.4289	0.2145	13.7857	0.0057
	Error	6	0.09333	0.01556		
	Total	8	0.5222			
Solvent Extract	Time	2	8400.7	4200.4	29.0791	0.0008
	Error	6	866.7	144.5		
	Total	8	9267.4			
Residual ^{14}C	Time	2	6318.7	3159.4	111.8535	$<$0.0001
	Error	6	169.5	28.3		
	Total	8	6488.2			
TNT in the Solvent extract	Time	2	9005.0	4502.5	32.9831	0.0006
	Error	6	819.1	136.5		
	Total	8	9824.0			

Table 18. Analysis of Variance Tables for the RDX Laboratory Composts

Parameter	Source of Error	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Probability
$^{14}CO_2$	Time	2	4808.2	2404.1	34.5751	0.0005
	Error	6	417.2	69.5		
	Total	8	5225.4			
H_2SO_4 Trap	Time	2	0.7356	0.3678	8.9459	0.0158
	Error	6	0.2467	0.0411		
	Total	8	0.9822			
Residual ^{14}C	Time	2	159.8	79.9	17.0779	0.0033
	Error	6	28.1	4.68		
	Total	8	187.9			
Solvent Extract	Time	2	12347.3	6173.6	86.5266	<0.0001
	Error	6	428.1	71.4		
	Total	8	12775.4			

recovered by 6 weeks was significantly different from both the 0 week and the 3-week recoveries. $^{14}CO_2$ recovered from the RDX laboratory composts at time zero, 3 weeks and 6 weeks were significantly different from each other. Analysis of the ^{14}C-recovery from solvent extracts of the TNT and RDX composts showed that recoveries at each of the sampling periods were significantly different from each other. Residual ^{14}C in the TNT composts was significantly different at each sampling period. Residual ^{14}C in the RDX composts at time zero was significantly different from the residual carbon recovered at 3 weeks and 6 weeks; however, the 3 week and 6 week recoveries were not significantly different from each other. Recovery of ^{14}C from the acid traps of the RDX composts showed that the 6 week recovery was significantly different from the 0 and 3 week recoveries; however, ^{14}C-recoveries from the acid traps at 0 and 3 weeks were not significantly different from each other.

C. Discussion and Conclusions Based on Laboratory Composting Data

Composting appeared to be an effective method of reducing TNT concentrations without the formation of the undesirable transformation products that are normally associated with TNT alteration in the environment or in biological systems. As the composting time increased, TNT levels in the composts were rapidly reduced as indicated by the recovery of ^{14}C-TNT in the solvent extracts (see Table 19). The solvent extractable TNT was reduced by half after three weeks of composting and after six weeks of composting less than 17% of the TNT was recovered on the average. The extract from one replicate of the six week compost did not contain detectable levels of TNT (less than 0.01%). The reduction in TNT was paralleled by a reduction in solvent extractable ^{14}C and an increase in the residual ^{14}C activity (Table 13). Degradation or transformation of TNT apparently resulted in the formation of products which are insoluble in benzene and/or are very strongly sorbed to the compost. Methanol acidified with acetic acid or made basic with sodium hydroxide was also ineffective at removing significant amounts of TNT by-products from the compost.

The reduction of TNT to mono- and diamino nitrotoluenes has been reported as the major route of TNT transformation in the environment (McCormick et al., 1976). In the composting process, however, only small amounts of these products are formed or they are rapidly converted or polymerized into other compounds. No TNT reduction products were found in the extracts from the 0 and 3 week composts. The TLC analysis of the six-week composts contained small amounts (0.9 to 2.0%) of ^{14}C in an elongated region adjacent to the origin. The diamino reduction product, 2,6-diamino-4-nitrotoluene, would have moved into this area but would be expected to be present as a more discrete round spot than was observed. However, it is possible that part of the ^{14}C-activity in the elongated region was present as 2,6-diamino-4-nitrotoluene. Two of three replicates of the six week composts did not contain detectable levels of the monoamino-DNT derivative. The extract from the third replicate had 1.1% of ^{14}C-activity tentatively identified as 4-amino-2,6-dinitrotoluene and 0.6% as 2-amino-4,6-dinitrotoluene.

Complete destruction of TNT by breaking the benzene ring does not appear to occur to any significant extent. Recovery of ^{14}C as $^{14}CO_2$ was negligible (less than 1%).

Table 19. Average Recovery of ^{14}C as TNT From Compost After 0, 3, and 6 Weeks of Composting

Length of Composting	% ^{14}C-TNT Recovered[1]
0 weeks	93.5
3 weeks	46.6
6 weeks	16.6

[1] ^{14}C-TNT recovered was determined by TLC analysis of compost extracts. The percentage of total ^{14}C-activity present in compost extracts as ^{14}C-TNT and as ^{14}C-labeled compounds other than TNT was determined by LSC.

Degradation of RDX in compost is rapid and appears to result in the complete destruction of the molecule. Recoveries of ^{14}C as $^{14}CO_2$ were in excess of 67% in individual replicates of the six-week composts. The average loss was 55.8%. The evolution of $^{14}CO_2$ was found to be inversely correlated to the recovery of ^{14}C-RDX in the solvent extract ($R = 0.9695$). It appears likely that when the RDX ring is attacked, the entire molecule is rapidly metabolized. Intermediate products, if any are formed, are readily assimilated by compost organisms and a large percentage of the RDX carbon is released as CO_2. ^{14}C-labeled compounds other than RDX were not found in the solvent extracts indicating that no build-up of solvent extractable intermediate products occurs.

Because of the rapid conversion of ^{14}C-RDX to $^{14}CO_2$, the plot of $^{14}CO_2$ versus time in Figure 6 can be used as an estimation of how RDX breakdown varies with time. Significant recoveries of $^{14}CO_2$ during the first four days of composting suggested that RDX degradation began almost immediately. During the first 11 to 15 days of composting, the rate of RDX breakdown was increasing. From the second week through the fourth week of composting, the rate of breakdown remained high. The $^{14}CO_2$ recoveries for the final two weeks of composting suggest a slow decline in the rate of RDX metabolism.

After 3 weeks of composting, the residual ^{14}C (^{14}C-activity in the compost material following solvent extraction) accounted for 13.5% of the total activity added to the compost (approximately 40% of the ^{14}C no longer solvent extractable as ^{14}C-RDX). The amount of residual ^{14}C in the compost after six weeks of composting was not significantly higher although RDX breakdown in the second three weeks of composting was substantial. Apparently secondary metabolism of any ^{14}C products formed from RDX was very rapid.

V. GREENHOUSE COMPOSTING

A. Greenhouse Compost Set-Up and Sampling

1. Soil Spikes

Lakeland soil (air dried and sieved) was used as the carrier for TNT. Two thousand grams of Lakeland soil were added to 500 mL of TNT solution (40% production grade TNT in acetone). The TNT concentration in the solution was verified by GC analysis. The mixture was stirred, maintained at ambient temperature in the dark and the acetone allowed to evaporate. Two replicates of TNT contaminated soil were prepared by the above procedures for the TNT greenhouse composts. Two thousand grams of the same Lakeland soil (no additions) were used in the control compost in the greenhouse.

A stock acetone solution of production grade RDX was assayed by gas chromatography and determined to contain 3.82% RDX. Two thousand grams of Lakeland soil were dosed with 2,620 mL of RDX solution (100.08 g RDX/2000 g soil) and treated as described above for the TNT soil spike.

2. Construction of Compost Chambers

Composting chambers were constructed of plywood. Dimensions of the chamber are given in Figure 9. The inside surfaces of the chamber were sealed with varnish and the outside surfaces were insulated with 3/4 inch styrofoam insulation. A lid was constructed of a double layer of the foam insulation.

Each of the composting chambers was placed in a glass 36 inch x 36 inch x 34 inch box. A layer of dry leaves was placed under and around the chamber for insulation. A bag of leaves or hay was placed on the top of each chamber for additional insulation.

Each compost box had provisions for pulling fresh air through the compost materials. Fresh air entered the top of the box and was drawn through the compost pile and out through a perforated polyethylene tube located beneath the compost pile. The polyethylene tubes were connected to the suction end of a blower. Air was drawn through the compost for a specified period during a ten minute cycle.

3. Set-Up of Greenhouse Composts

Duplicate greenhouse composts for each explosive (RDX and TNT) were set-up in a manner similar to the laboratory scale composts. A single untreated (i.e. no explosives added) compost served as a control. The compost size was approximately 10 Kg. The soil accounted for 2000 g of the mass. The bulk of the compost was a 50:50 mixture of chopped alfalfa hay and horse feed. A portion of the hay (approximately 500 g) was layered in the bottom of the composting chamber to soak up leachate. The remaining hay and horsefeed were mixed and watered before the treated or uncontaminated soil was mixed in. A small amount of seed compost or horse manure was slurried with water and added to initiate the composting process.

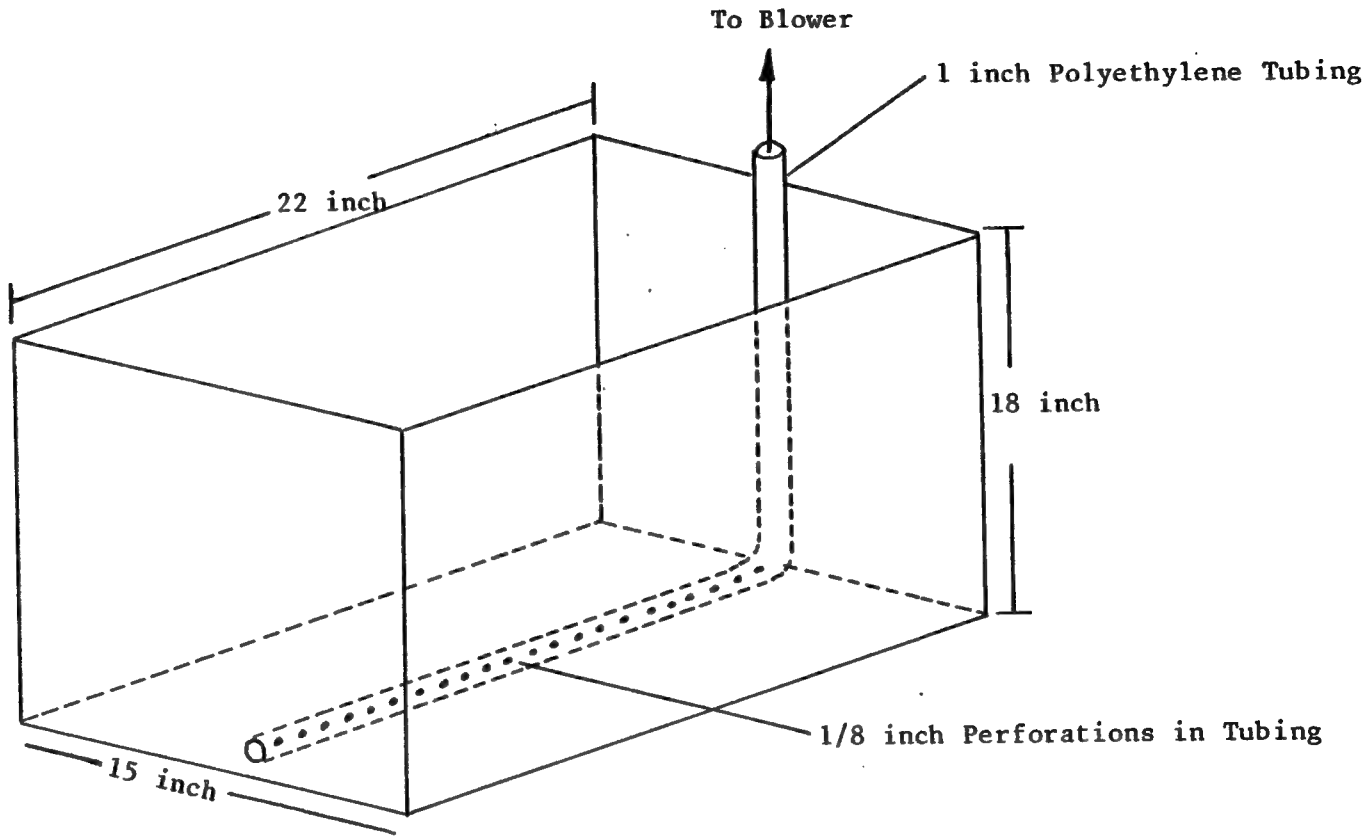


Figure 9. Schematic of Greenhouse Compost Chamber

The dry weights of the initial compost ingredients are listed in Table 20. Some of the composts required several manure additions to start the compost. During six weeks of composting, hay and horse feed were added to the compost piles to maintain elevated temperatures. All additions and removals were corrected for the moisture contents of the materials. The range of moistures was determined by drying at 80°C for 24 hours. The moisture contents of the composts, the hay, the horse feed and seed materials are given in Table 21.

4. Sampling Procedure

A chopped hay and horsefeed compost is a relatively homogeneous mass when viewed as a whole. However, small subsamples of such a compost (of a size suitable for extraction to determine the RDX and TNT concentrations) are not homogeneous, but may vary greatly between samples. Therefore, the initial sampling of both the RDX and TNT composts (greenhouse scale) was designed to provide information on the effect of subsample size on the accuracy of determining the concentration of explosives in the compost.

Three 20 g and three 50 g (wet weight) subsamples were removed from each of the two TNT and RDX replicates. Subsamples were obtained by mixing the compost and removing a number of grab samples. These samples were combined and mixed, and then the 20 and 50 g subsamples were removed from the sample. Several additional samples were also removed for moisture determination. The remaining sample of compost was mixed back into the compost. The subsamples were extracted with acetone for RDX analysis and with benzene:methanol for TNT analysis. The extracts were analyzed by GC as described in Section IV and Appendices B and C. The results are presented in Table 22. The variability between subsamples for both explosives is high, as indicated by the standard deviation. The variability in the RDX samples is particularly high. This variability is the result of crystallization of RDX in the treated soil. Soil particles were cemented together when the RDX crystallized resulting in relatively large aggregates. Attempts to crush these aggregates were only partially successful, therefore, RDX could not be as evenly dispersed in the compost as the TNT. A one-way analysis of variance, Model II, was utilized to find which subsample size gave a more precise estimate of the explosive concentration. The ANOVA's for both RDX and TNT are presented in Table 23. The F ratios were not significant for either RDX or TNT indicating that the sample size did not significantly influence the precision of determining TNT or RDX levels in compost. The standard deviation for the 50 g subsamples was substantially lower than that for the 20 g samples, therefore, 50 g subsamples with four subsamples per replicate were used in all subsequent samplings.

B. Results

1. Routine Monitoring of Greenhouse Composts

Three thermocouples were inserted in the center of each compost: one 11.5 to 13 cm (4.5-5 inches) from the bottom; one 23 to 25 cm (9-10 inches) from the bottom and one 34 to 38 cm (13.5-15 inches) from the bottom. The

Table 20. Greenhouse Compost Ingredients

Compost Box	Weight in Grams						
	Explosive	Soil	Hay	Horse Feed	Seed Compost	Manure	Total
1	0	2000	4815	3900	121	0	10836
2	100 RDX	2000	3900	3900	0	465	10265
3	100 RDX	2000	3900	3900	0	465	10265
4	200 TNT	2000	3900	3900	121	0	9921
5	200 TNT	2000	4815	3900	121	0	10836

Table 21. Moisture Contents of Greenhouse Compost and Compost Components .

Material	% Moisture
Hay	7.5 - 8.5
Horse Feed	8.4 - 12.3
Seed Compost	67.8
Horse Manure	50.9 - 61.3
Compost - 0 week	52.2 - 61.6
Compost - 3 week	51.6 - 72.0
Compost - 6 week	63.2 - 67.3

Table 22. RDX and TNT Concentrations in Greenhouse Composts at Time Zero Sampling

	Subsample Size (wet wt)	Replication	Concentration of Explosive (ppm)		
RDX	20 g	1	9794	$\bar{X}^*$	12606
			9297	S	5307
			18727		
		2	12839	$\bar{X}$	8629
			7081	S	3688
			5967		
	50 g	1	9097	$\bar{X}$	8933
			7435	S	1423
			10267		
		2	15434	$\bar{X}$	10198
			8659	S	4661
			6502		
TNT	20 g	1	18154	$\bar{X}$	17916
			20789	S	3000
			14804		
		2	20431	$\bar{X}$	20695
			22574	S	1762
			19080		
	50 g	1	19128	$\bar{X}$	21441
			20694	S	2764
			24502		
		2	20302	$\bar{X}$	20053
			18279	S	1664
			21578		

*$\bar{X}$ - arithmetic mean

S - standard deviation

Table 23. Analysis of Variance Examining Subsample Size for Greenhouse Scale RDX and TNT Composts

RDX

Source of Error		Degrees of Freedom	Sums of Squares	Mean Square	F Ratio	Probability
Among subsample sizes		1	116704	1106704	0.25417	0.664
Within subsample size		2	8708377	4354189		
Total		3	9815081			
20 g subsample	$\bar{X}$	10618				
	S	2812				
50 g subsample	$\bar{X}$	9566				
	S	894				

TNT

Source of Error		Degrees of Freedom	Sums of Squares	Mean Square	F Ratio	Probability
Among subsample sizes		1	2077922	2077922	0.86137	0.451
Within subsample size		2	4824693	2412346		
Total		3	6902615			
20 g subsample	$\bar{X}$	19306				
	S	1965				
50 g subsample	$\bar{X}$	20747				
	S	981				

$\bar{X}$ = arithmetic mean
S = standard deviation

middle thermocouple is plotted as a function of time in Appendix D. In the TNT and control composts, the temperatures at the bottom of the compost were slightly cooler on the average than the temperatures in the middle. The top thermocouple readings were within 2°C of the temperatures in the middle of the compost. The temperatures at the bottom of the RDX composts were consistently as warm as, or slightly warmer than, the temperatures in the middle of the box. The thermocouple readings from the top of the compost were consistently cooler than the temperature in the middle of the compost.

The air removed from the composts by the aeration system was sampled weekly for GC analysis of its O_2 and CO_2 contents. The results are presented in Appendix E. The O_2 levels ranged from 4.5% to 20.3% of the air. At no time during the composting period did analysis indicate that the composts had become anaerobic.

2. Compost Extraction and Analysis

The RDX composts were subsampled after 0, 3, and 6 weeks of composting. Because of the rapid decrease in extractable TNT, the TNT composts were subsampled after 0, 3 and 4 weeks of composting. The subsamples were extracted with acetone for RDX recovery and benzene:methanol for TNT recovery. Quantification of the explosives was by GC analysis as described in Section II and Appendices B and C. Concentrations of explosives in the composts are presented in Tables 24 and 25. Subsamples for the control compost were spiked with standard solutions of explosive at each sampling time, extracted and analyzed in the same manner as the experimental composts. Results of the quality control analyses are presented in Tables 26 and 27.

The recoveries of TNT and RDX from the compost were analyzed in a one way analysis of variance. The RDX data did not lack homogeneity of variance at the 5% level of probability according to Cochran's Test (Chemical Rubber Company Handbook, 1968). There was insufficient data to test the homogeneity of variance of the TNT results, therefore, no data transformation was used. The significance testing was at the 5% level. Where significant differences were indicated by the analysis of variance, the Student-Newman-Kuels Multiple Range Test was used to separate means. The results of the analysis of variance are presented in Table 28.

C. Discussion and Conclusions

Composting of TNT on a greenhouse scale resulted in rapid disappearance of solvent extractable TNT from compost. Analysis of solvent extracts at three weeks showed that TNT concentrations were below the detection limit indicating that the process by which the TNT concentration is reduced during composting occurred more rapidly in the greenhouse compost than in the laboratory scale composts. Greenhouse compost temperatures were variable but, in general, were higher than the temperatures recorded for the laboratory composts. It is possible that the elevated temperatures enhanced the disappearance of TNT from the compost material.

Table 24. TNT Concentration in Greenhouse Compost Material

Sample	µg/g in Compost		
	T_o Week	T_3 Week	T_4 Week
Box 1 (control)	<16.9	<16.9*	<16.9
Box 4	19,678	<16.9	<16.9
Box 5	20,404	<16.9	<16.9

*Detection Limit for Quantification of TNT from Compost was 16.9 µg/g

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Table 25. RDX Concentration in Greenhouse Compost Material

Sample	µg/g in Compost		
	T_o Week	T_3 Week	T_6 Week
Box 1 (control)	ND*	ND*	ND*
Box 2	9,240	3,284	3,142
Box 3	9,414	5,093	1,277

*Detection Limit for Quantification of RDX from Compost was 794.7 µg/g

Table 26. Quality Control: TNT Compost Sampling

QC Sample	µg/g Target	Found - T_o	Found - T_3
A	30.7 ppm	40.1	41.3
B	77.0 ppm	75.8	77.8
C	153.0 ppm	175.5	143.5
D	460.0 ppm	500	467.8

QC Sample	µg/g Target	Found - T_4
A	4.6	4.8
B	9.2	7.0
C	25.2	26.0
D	50.4	50.0

Table 27. Quality Control: RDX Compost Sampling

QC Sample	Time	µg/g	
		Target	Found
A	zero	1180	922
B		2359	2027
C		4444	4719
D		9196	9438
A	3 week	1569	1245
B		3138	2640
C		6275	5040
D		12551	9480
A	6 week	1170	740
B		2340	2404
C		4680	4345
D		9359	9329

Table 28. Analysis of Variance for TNT and RDX Levels in Greenhouse Composts

Parameter	Source of Error	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	Probability
TNT	Time	2	55467806	27733903	24.6496	0.01374
	Error	3	3375371	1125124		
	Total	5	58843177			
RDX	Time	2	534614101	267307051	3042.9052	0.00001
	Error	3	263538	87846		
	Total	5	534877639			

Breakdown of RDX in the greenhouse compost was initially much more rapid that that observed in laboratory composts. After three weeks of composting, RDX levels in the greenhouse scale composts were reduced by 61%, compared to an average of 39% reduction observed in the laboratory composts. It should be noted that the RDX concentration in the greenhouse compost after three weeks is neither corrected for additions of cmposting materials nor for the loss of compost mass through microbial respiration and is thus an approximation. The entire compost must be weighed to calculate mass reductions via respiration. This measurement could only be made at the conclusion of the experiment.

Total reduction of RDX by composting for six weeks averaged 82% and 81% from the greenhouse and laboratory compost, respectively. The close agreement between the greenhouse and laboratory composts indicates that bench scale composts would be accurate in predicting the metabolism of RDX in large scale composts. The greenhouse composts generally composted at higher temperatures. This difference in temperature did not have any apparent effect on RDX breakdown.

Collectively the results from the laboratory and greenhouse composts indicate that both RDX and TNT concentrations are rapidly decreased by composting. The laboratory composting equipment and conditions used in this study were sufficient to provide a good estimate of the breakdown of explosives in larger scale composts. These conditions can likely be altered to improve the accuracy of the bench scale composts for use in predicting what occurs in full size composts.

VI. LEACHATE STUDY

The methodology used in the leachate studies is outlined in Table 29 and discussed along with the results in the following paragraphs.

A. Preliminary Study

The objective of the preliminary study was to measure the maximum water holding capacity of composted materials. This study was necessary to determine appropriate procedures to be used in obtaining aqueous extracts of the compost. Evaluation of alternative methods for clarifying the aqueous extract was included in this study.

1. Water Holding Capacity

Several techniques for determination of water holding capacity of compost were attempted. The compost was sampled and percent moisture determined by drying at 60°C for 24 hours. Compost samples were weighed onto filter paper or paper towels which were supported by metal screens. Other samples were wrapped in a variety of materials; cheesecloth (3 layers), lens paper (single thickness) and Kimwipes (single thickness). All samples were saturated with tap water for at least 45 minutes and allowed to drain. Time required for complete draining was excessive, requiring more than 24 hours in some cases. The volume of water retained by the compost was corrected for the water absorbed by the support materials (filter paper, towels, etc.); however, the results were too variable to be considered reliable.

As an alternative method, compost was mixed with a known volume of water in a 100 mL graduated cylinder and allowed to absorb water for one hour. The compost was then compressed into the bottom of the cylinder and the free water decanted. The volume of free water was measured and recorded. Two composts were used in these studies: a three-week old chopped hay and horsefeed compost and a similar compost that was started approximately nine months prior to use. The results of these tests are summarized in Table 30. The water retention ratios observed were relatively consistent. The three week old compost was not as putrefied as the nine month old material and therefore, had a much lower water holding capacity. Composts to be extracted in the leachate study to estimate leachable TNT, RDX and metabolites will be up to six weeks old. The water retention of a six week old compost will be between that determined for the 3 week and the 9 month old composts. Therefore, a 6:1 ratio of water to solids was selected to insure a sufficient volume of sample for analysis.

Table 29. Summary of Leachate Compost Studies

1) Preliminary Study - determine water retention capacity of compost at varying stages of composting.
2) Compost - 50 g (dry weight) hay and horsefeed compost dosed at 1% RDX or 1% TNT.
3) Compost Procedure -
   a. Composting carried out in a 55°C incubator
   b. Compost continuously aerated with humidified and warmed CO_2 free air
   c. Two replicate composts sacrificed after 0, 3 and 6 weeks of composting for aqueous extraction to determine TNT and RDX losses in leachate.

Table 30. Absorption of Water by Composted Material

Compost	Compost Weight (g) Wet	Dry*	H_2O Added (mL)	H_2O Retained** (mL)	Ratio of Liquid to Solid
9 month	20.88	3.88	10.0	23.21	7.2
	20.00	3.08	20.0	22.12	7.2
	20.51	3.16	30.0	21.11	6.7
	20.45	3.15	40.0	22.25	7.1
3 week	21.9	10.42	69.7	40.43	3.9

*Dried 24 hours at 60°C

**Total water retained in compost; includes water already in compost (wet weight - dry weight) plus any additional water absorbed.

2. Clarification of Aqueous Extract

Clarification of the extract obtained from the compost was attempted by filtering through several types of filter paper, glass wool, sand, and by centrifugation. It was found that filtration through glass wool to remove the larger particulates, followed by centrifugation to remove most of the remaining particulates, followed by filtration through Whatman No. 42 filter paper was the most efficient method for removing the particulate matter from solution.

B. Leachate Study

Twelve 50 g composts were prepared with TNT or RDX added as 1% of the compost to each of six flasks. The moisture content of chopped alfalfa hay, Purina Sweetena horsefeed and seed compost was determined by drying at 80°C for 24 hours. The hay (18.5 g dry weight), horse feed (18.5 g dry weight) and seed compost (3 g dry weight) were combined with Lakeland soil previously treated with TNT or RDX. Methods of treating the soil and mixing the compost were as described in the Preliminary TNT Laboratory Compost section of this report. The final water content of the compost was adjusted to 60% (wet weight basis). The composts were placed in an incubator at 55°C and aerated as were the control composts in the preliminary TNT compost study (see Figure 2).

The temperatures of the composts were monitored daily. Individual compost temperatures ranged from 50 to 62°C. The average temperatures of the TNT and RDX composts and the average air temperature of the incubator are plotted against time in Figure 10. The TNT composts averaged 2.3°C higher than the air temperature in the incubator. The RDX compost temperatures averaged 4.8°C higher than the incubation.

Duplicate samples of the compost were sacrificed at time zero and after 3 weeks and 6 weeks of incubation. The samples were extracted for 20 hours with distilled water to simulate a worst case example of leaching by rain in a field operated compost.

C. Results

Analysis of the RDX compost leachate at time zero indicated that 7.4% of the RDX (approximately 124 ppm) was leached into the water extract. A significant decrease in RDX leaching was observed after composting with 3.2% (approximately 52.5 ppm) detected at 3 weeks and 0.8% (13 ppm) after six weeks of incubation.

Analysis of the TNT compost leachate at time zero showed that TNT was not leached into the water extract in detectable amounts from fresh compost materials. Leachate analysis at three weeks of incubation showed that 5.9% of the TNT was present in the leachate (approximately 98 ppm), indicating that TNT is more readily extracted with aqueous or polar solutions from composted material than from the fresh compost materials. After six weeks of composting, the TNT leachate contained 0.08% of the TNT (1.4 ppm)

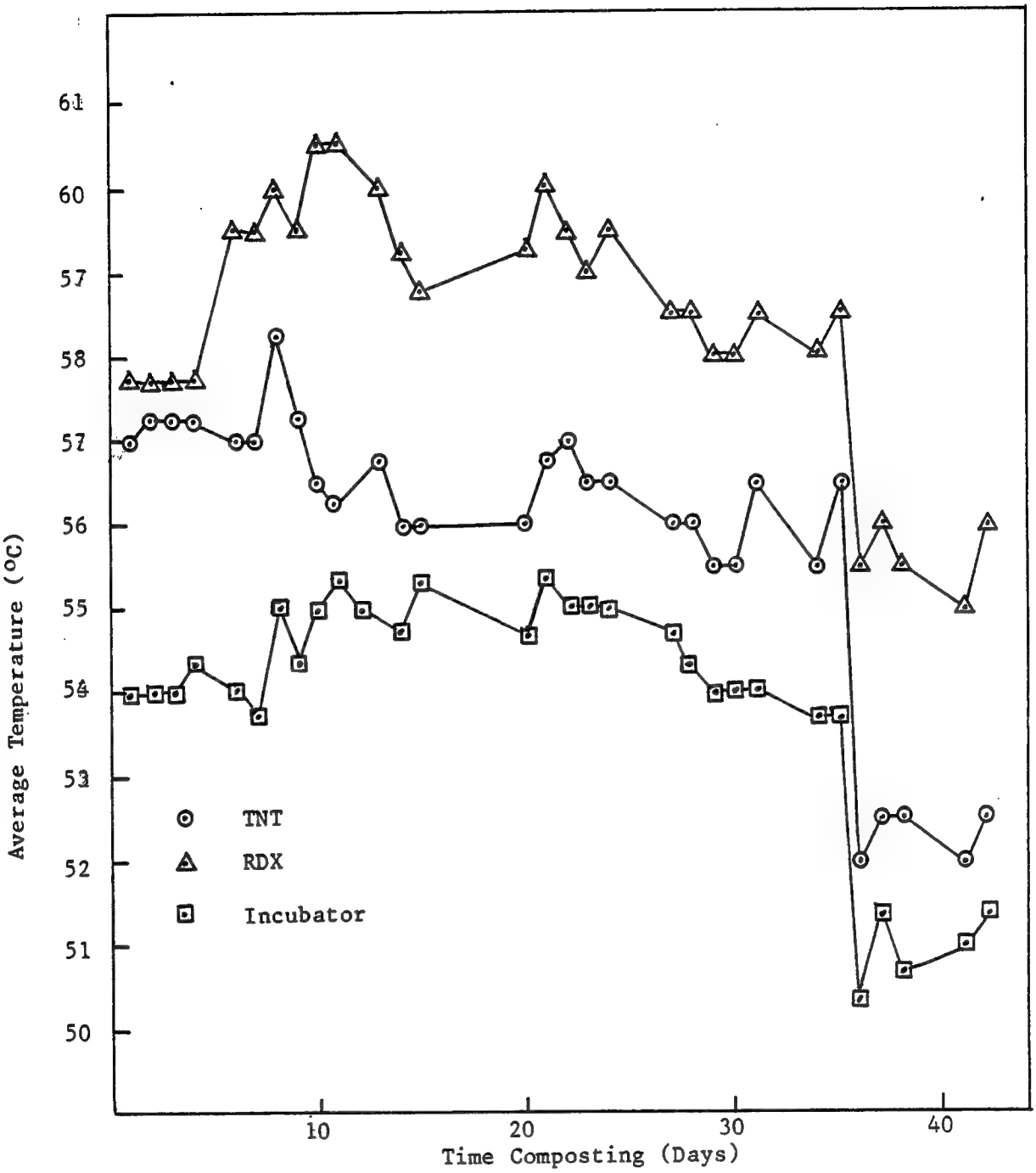

Figure 10 Comparison of Compost Temperatures for Leachate Study

D. Conclusions

The leachate study was performed under conditions designed to illustrate a "worst-case" example. The soil containing the TNT or RDX was a sand with less than 5% clay and silt, and approximately 1% organic matter. Such a soil is expected to have a relatively low capacity to adsorb and retain organics such as TNT or RDX. The twenty hour extraction at room temperature prior to removal of the aqueous leachate would likely result in TNT and RDX concentrations far greater than would normally be found following rainfall and leaching from an outdoor compost pile. The decrease in RDX concentration in the leachate following composting corresponds to the biodegradation of this explosive during the incubation period.

The very small amounts of TNT found in the aqueous extracts of the TNT composts indicate that TNT is not readily extracted from fresh compost materials by polar solvents. During the initial three-week composting period, the adsorption of TNT to the compost materials appears to be altered with an increased quantity of TNT leaching into the extract. The subsequent decrease in TNT concentrations in the 6-week leachates corresponds to the disappearance of TNT during the incubation period.

VII. REFERENCES

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Chemical Rubber Company (1968), Handbook of Tables for Probability and Statistics, CRC, Cleveland, Ohio. Cochran's Test for the Homogeneity of Variances, p. 325-327.

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Won, W.D.; R.J. Heckley; D.G. Glover and J.C. Hoffsommer (1974), "Metabolic Disposition of 2,4,6-trinitrotoluene," Appl. Micro., 27(3), p. 513-516.

APPENDIX A. SYNTHESIS OF ^{14}C-LABELED RDX

Two mL of concentrated NH_4OH are slowly aded with mixing to 2 mL of 40% formaldehyde (containing 250 μ Ci of ^{14}C-formaldehyde) to form a hexamine solution.

To the hexamine solution, 0.45 mL of 70% nitric acid is slowly added with mixing in an ice-salt bath at 5-15°C. An additional 0.3 mL nitric acid is added to cause precipitation. The solution is maintained at 5°C for an additional 15 minutes. The sample is then centrifuged, the liquid drawn off and discarded. The resulting crystals are dried in a vacuum oven at room temperature. A yield of approximately 1 g hexamethylenetetramine-dinitrate is obtained.

To the dried salt, 0.623 g finely divided ammonium nitrate is added and thoroughly mixed.

0.7 mL of 98% nitric acid is added slowly to a test tube containing 2.65mL of acetic anhydride cooled to 5-15°C in an ice-salt bath.

In another tube, a small amount of the solid and the liquid mixtures are added together and quickly heated in a water bath to 70-80°C. Small amounts of the solid and liquid mixtures are added until all of the solid and liquid have been used. The mxiture is allowed to heat for an additional 15 minutes and then cooled to 15°C to precipitate the RDX.

The cooled mixture is centrifuged, the liquid drawn off, the crystals rinsed with 2 mL cold water and again centrifuged. The liquid is drawn off, and the RDX crystals dried in a vacuum oven at room temperature.

APPENDIX B. ANALYSIS OF TNT IN COMPOST - QUANTITATIVE

1. APPLICATION

Method used to determine the concentration of TNT in compost.

A. Tested Concentration Range: (µg/g)

5.6 µg/g to 110.8 µg/g

B. Sensitivity:

1091 area units/pg based on a 35.4 pg injection

C. Detection Limit: (µg/g)

16.9 µg/g

D. Interferences: Interferences were encountered which could be attributed to compost components, the presence of phthalate esters or their plasticizers.

E. Analysis Rate: Extraction requires 1.5 hours to complete. One analyst can extract and analyze 12 samples per 8-hour day.

2. CHEMISTRY

$C_7H_5N_3O_6$	Toluene, 2,4,6-Trinitro-
CAS RN	118-96-7
Melting Point:	80.75°C
Boiling Point:	240°C (explodes)

Hazards: Use caution in handling this compound; explosive and toxic hazards exist.

3. APPARATUS

A. Instrumentation:

Gas Chromatograph - Hewlett-Packard 5880A with computer controller and integrator, autoinjector, and electron capture detector.

B. Parameters:

Column - 1.5% OV17/1.95% OV210 on 80/100 Anakrom Q in a 2 mm I.D., 0.125 in O.D. by 6 ft. glass column
Temperature - injection port - 210°C
oven - 180°C
detector - 300°C
Temperature Programming - isothermal
Carrier Gas - nitrogen at 28 cc/min.
Detector - electron capture
Injection Volume - 2 µL
Retention Time - 3.2 min.

C. Glassware/Hardware:

Volumetric Flask - 2 mL (2)
Volumetric Flask - 50 mL (1)
Volumetric Flask - 25 mL (3)
Volumetric Pipets - 5 mL (2)
Volumetric Pipets - 1 mL (3)
Volumetric Pipets - 1/2 mL (1)
Filter Paper, Fisher qualitative 42
Glass Funnels - 9 cm (6)
Glass Graduated Cylinders - 500 mL (6)
1 Quart Mason Jars (6)
Finn Pipets (adjustable) - 200 - 1000 µL
Finn Pipetts (adjustable) - 50 - 200 µL
Finn Pipetts (adjustable) - 5 - 50 µL
GC Autosampler Vials with Teflon Inserts (10)
Aluminum Foil
Waterbath - 37°C
Refrigerator
Test tubes, glass (6)

D. Chemicals:

TNT "SARM"- PA 360, Lot #268
Benzene, certified (Fisher Scientific)
Methanol, certified (Fisher Scientific)

4. STANDARDS

A concentrated stock solution of TNT is prepared by weighing out the following amount of SARM material into a volumetric flask and bringing to volume with benzene.

14.2 mg in 100 mL = 142 mg/L (I)

The volumetric flask is wrapped in aluminum foil and stored in the refrigerator until needed. Storage time should not exceed two months.

A. Calibration Standards: Calibration standards are prepared from the stock solution by dilution with benzene according to the following scheme:

.5 ml of I to 20 mL	=	3.55 mg/L (II)
2 mL of II to 10 ml	=	710 µg/L (III)
1 mL of II to 20 mL	=	177 µg/L (IV)
1 mL of III to 10 mL	=	71 µg/L (V)
1 mL of IV to 10 mL	=	17.7 µg/L (VI)

B. Control Spikes:

Control spikes are prepared as follows:

20 mg TNT SARM in 50 mL benzene = 400 µg/mL (A). Compost weight is 20 grams.

10 DL	5.54 mL of A	110.8 µg/g
5 DL	2.75 mL of A	55.0 µg/g
2 DL	1.10 mL of A	22.0 µg/g
1 DL	0.55 mL of A	11.0 µg/g
.5 DL	0.28 mL of A	5.6 µg/g
Blank	0 mL of A	0 µg/g

5. PROCEDURE

Four grams of Lakeland sand are weighed into each of six 50 mL beakers. Each beaker of sand is dosed with the appropriate amount of TNT stock. After the spike, each beaker is covered with aluminum foil and placed in the dark at room temperature overnight.

Each dosed soil is added to 16 grams compost (dry weight) and mixed in one quart Mason jars. After mixing, the jars are wrapped in foil, and placed in the dark at room temperature for one hour.

The extraction is carried out with 160 mL benzene:methanol (75:25). Warm extractant, 160 mL, is added to each Mason jar and the jars are placed in a 37°C waterbath. All jars are agitated at 5 minute intervals. Jars are removed from the waterbath after 30 minutes. The liquid extract from each jar is filtered through filter paper in a glass funnel. The filtrate is collected in glass test tubes.

The samples are diluted for analysis by the following procedure:

DL	mL Extract	mL Benzene	Dilution
0	1	1	1:2
0.5	1	1	1:2
1.0	5	20	1:5
2.0	5	45	1:10
5.0	1	24	1:25
10.0	0.5	24.5	1:50

All dilutions are made using volumetric pipets and volumetric flasks.

TNT analysis by GC may be accomplished with flame ionization (FID) or electron capture (EC) detectors. The detection limit with FID is 50 ppm and requires concentration of the compost extract for analysis. Concentration of the compost extract before analysis is not feasible because of the interferences present in the extract. The range for detection of TNT with EC is 15-500 ppb. Thus, the compost extracts must be diluted to fall within this analytical range. Dilution of the extracts decreases the interferences caused by the compost components, phthalate esters or their plasticizers.

Inject 2 µL of the diluted extract onto the GC column in duplicate.

Run standards singly at the beginning and end of each run

Plot peak area versus ppb injected to obtain standard curves for TNT.

6. CALCULATIONS

The concentration of explosive (ppb) in the sample is read directly from the standard curve. The apparent concentration of explosive in the compost is calculated from the formula given below:

$$\text{Concentration (ppm)} = \text{ppb} \times \frac{\text{120 mL extract x .001 x reciprocal of extract dilution}}{\text{g dry weight compost (50 g wet weight)}}$$

7. REFERENCE

Lindner, V. (1980), "Explosives and Propellants," Kirk-Othmer Encyclopedia Chemical Technology, 3rd edition, John Wiley and Sons, NY, 9:561-671.

TNT IN COMPOST

Target Concentration	Days 1	2	3	4
µg/g				
Blank 0	0.30	0.37	0.08	0.28
0.5X 5.6	3.55	3.89	2.80	3.60
X 11.0	9.15	8.37	6.45	7.59
2X 22.0	14.70	17.16	15.66	22.50
5X 55.0	45.00	45.45	37.80	52.50
10X 110.8	108.30	95.70	91.50	89.70

Target Concentration	Average Found Value	Standard Deviation	Percent Imprecision	Percent Inaccuracy
µg/g				
Blank 0	.26			
0.5X 5.6	3.46	.47	13.48	-38.2
X 11.0	7.89	1.15	14.6	-28.3
2X 22.0	17.51	3.48	19.9	-20.4
5X 55.0	45.19	6.00	13.3	-17.8
10X 110.8	96.30	8.39	8.7	-13.1

SUM(X(i))= 814.0000
SUM(X(i)^2)= 63041.0000
SUM(Y(i)^2)= 47145.3662
SUM(X(i)*Y(i))= 54276.9480

TNT IN COMPOST

X= 1.1381 Y + 1.5557 CORR. COEF.= 0.9930
Y= 0.8786 X + -1.3669 CORR. COEF.= 0.9930

LINE	TC	C(FOUND)	C(CALC)	DELTA	GROUP
1	0.0000	0.3000	-1.3669	1.6669	0.0000
2	0.0000	0.3720	-1.3669	1.7389	0.0000
3	0.0000	0.0840	-1.3669	1.4509	0.0000
4	0.0000	0.2760	-1.3669	1.6429	0.0000
5	5.5000	3.5520	3.4656	0.0864	1.0000
6	5.5000	3.8880	3.4656	0.4224	1.0000
7	5.5000	2.7960	3.4656	-0.6696	1.0000
8	5.5000	3.6000	3.4656	0.1344	1.0000
9	11.0000	9.1500	8.2980	0.8520	2.0000
10	11.0000	8.3700	8.2980	0.0720	2.0000
11	11.0000	6.4500	8.2980	-1.8480	2.0000
12	11.0000	7.5900	8.2980	-0.7080	2.0000
13	22.0000	14.7000	17.9629	-3.2629	3.0000
14	22.0000	17.1600	17.9629	-0.8029	3.0000
15	22.0000	15.6600	17.9629	-2.3029	3.0000
16	22.0000	22.5000	17.9629	4.5371	3.0000
17	55.0000	45.0000	46.9577	-1.9577	4.0000
18	55.0000	45.4500	46.9577	-1.5077	4.0000
19	55.0000	37.8000	46.9577	-9.1577	4.0000
20	55.0000	52.5000	46.9577	5.5423	4.0000
21	110.0000	108.3000	95.2822	13.0178	5.0000
22	110.0000	95.7000	95.2822	0.4178	5.0000
23	110.0000	91.5000	95.2822	-3.7822	5.0000
24	110.0000	89.7000	95.2822	-5.5822	5.0000

STANDARD ERROR OF ESTIMATE (Sxy) = 4.2043
N= 24.0000 n= 1.0000
TOTAL N= 24.0000
t= 1.7170
% BASED ON TOTAL N
UPPER CONFIDENCE LIMIT AT(X=0) = 6.1147

upper confidence line at X= 5.5000 is 10.9134
lower confidence line at X= 5.5000 is -3.9822
STANDARD DEVIATION AT X= 5.5000 is 0.4662
PERCENT INACCURACY AT X= 5.5000 IS -37.1091
PERCENT IMPRECISION AT X= 5.5000 IS 13.4790
MEAN FOUND AT X= 5.5000 IS 3.4590

upper confidence line at X= 11.0000 is 15.7179
lower confidence line at X= 11.0000 is 0.8781
STANDARD DEVIATION AT X= 11.0000 is 1.1520
PERCENT INACCURACY AT X= 11.0000 IS -28.2727
PERCENT IMPRECISION AT X= 11.0000 IS 14.6013
MEAN FOUND AT X= 11.0000 IS 7.8900

upper confidence line at X= 22.0000 is 25.3447
lower confidence line at X= 22.0000 is 10.5811
STANDARD DEVIATION AT X= 22.0000 is 3.4805
PERCENT INACCURACY AT X= 22.0000 IS -20.4318
PERCENT IMPRECISION AT X= 22.0000 IS 19.8827
MEAN FOUND AT X= 22.0000 IS 17.5050

upper confidence line at X= 55.0000 is 54.3695
lower confidence line at X= 55.0000 is 39.5458
STANDARD DEVIATION AT X= 55.0000 is 6.0042
PERCENT INACCURACY AT X= 55.0000 IS -17.8409
PERCENT IMPRECISION AT X= 55.0000 IS 13.2873
MEAN FOUND AT X= 55.0000 IS 45.1875

upper confidence line at X= 110.0000 is 103.2066
lower confidence line at X= 110.0000 is 87.3578
STANDARD DEVIATION AT X= 110.0000 is 8.385-
PERCENT INACCURACY AT X= 110.0000 IS -12.4545
PERCENT IMPRECISION AT X= 110.0000 IS 8.7079
MEAN FOUND AT X= 110.0000 IS 96.3000

DETECTION LIMIT = 16.9332	ug/g

APPENDIX C. ANALYSIS OF RDX IN COMPOST - QUANTITATIVE

1. APPLICATION

Method used to determine the concentration of RDX in compost.

A. Tested Concentration Range: (µg/g)

630 to 12600 µg/g

B. Sensitivity:

7150 area units/ng based on a 23.4 ng injection

C. Detection Limit: (µg/g)

794.7 µg/g

D. Interferences: Major interferences in RDX analysis were encountered due to the acetone extraction of many compost components as well as RDX. A number of the components in the extract eluted from the GC column at approximately the same time as RDX. Separation of these components from RDX to allow quantitation is extremely difficult.

E. Analysis Rate: Extraction requires 3 hours to complete. One analyst can extract and analyze 12 samples per 8-hour day.

2. CHEMISTRY

$C_3H_6N_6O_6$	Hexahydro-1,3,5-trinitro-1,3,5-triazine
CAS RN:	121-82-4
Melting Point:	204°C
Boiling Point:	Not available

Hazards: Use caution in handling RDX; potential explosive and toxic hazards exist.

3. APPARATUS

A. Instrumentation:

Gas Chromatograph - Hewlett-Packard 5880A with computer controller and integrator, autoinjector and electron capture detector.

B. Paramaters:

Column: 10% SE30 on 80/100 Supelcoport in a 2 mm I.D., 0.25 in O.D. by 2 ft. glass column
Temperature: injection port - 210°C
oven - 180 to 210°C
detector - 340°C
Temperature Programming: 10°C/min.
Carrier Gas: nitrogen at 30 cc/min.
Detector: electron capture
Injection Volume: 2 µL
Retention Time: 3.90 min.

C. Glassware/Hardware:

Glass filter flasks (6)
Glass beakers, 50 mL (6)
Filter paper, Fisher qualitative medium #42
Buchner funnel, plastic, 9 cm (4)
One quart Mason jars (6)
Finn pipette adjustable, 200-1000 µL
Finn pipette adjustable, 50-200 µL
Finn pipette adjustable, 5-50 µL
Volumetric pipets, 1 mL (9)
Volumetric pipet, 2 mL (1)
Volumetric pipets, 5 mL (4)
Volumetric flasks, 100 mL (4)
Volumetric flasks, 10 mL (10)
Graduated cylinders, 500 mL (6)
Water bath, 37°C
Aluminum foil
Refrigerator

D. Chemicals:

RDX "SARM", Lot #HOL475-1, PA 361
Acetone, ACS certified (Fisher Scientific)
Benzene, ACS certified (Fisher Scientific)
Anhydrous Sodium Sulfate, ACS certified (Fisher Scientific)

4. STANDARDS

A concentrated stock solution of RDX is prepared by weighing out the following amount of SARM material into a volumetric flask and bringing to volume with acetonitrile:

93.44 mg to 100 mL = 934.4 mg/L (I)

The volumetric flask is wrapped in aluminum foil and stored in the refrigerator until needed.

A. Calibration Standards: Calibration standards are prepared from the stock solution by dilution with benzene according to the following scheme:

5 mL of I to 100 mL	=	47.6 mg/L (II)
2.5 mL of I to 100 mL	=	23.4 mg/L (III)
5 mL of III to 10 mL	=	11.7 mg/L (IV)
1 mL of II to 10 mL	=	4.8 mg/L (V)
1 mL of III to 10 mL	=	2.3 mg/L (VI)
1 mL of IV to 10 mL	=	1.2 mg/L (VII)

B. Control Spikes:

Control Spikes are prepared as follows:

4.2 g of RDX to 100 mL acetone = 42,000 mg/L (A)

Compost weight is 20 g (dry weight)

10 DL	6.0 mL of A	=	12600 µg/g
5 DL	3.0 mL of A	=	6300 µg/g
2 DL	1.2 mL of A	=	2520 µg/g
1 DL	600 µL of A	=	1260 µg/g
0.5 DL	300 µL of A	=	630 µg/g
Blank	0 mL of A	=	0 µg/g

5. PROCEDURE

Four grams of Lakeland sand are weighed into each of six 50 mL beakers. Each beaker of sand is dosed with the appropriate amount of RDX stock. Each beaker is covered with aluminum foil and placed in the dark at room temperature overnight.

Each dosed soil is added to 16 grams (dry weight) compost (50 g wet weight) and mixed in one quart Mason jars. After mixing, the jars are wrapped in foil and placed in the dark at room temperature for one hour.

Three extractions are carried out with acetone. Warm acetone, 160 mL, is added to each Mason jar and the jars are placed in a 37°C water bath. All jars are agitated at 0, 10 and 20 minutes. Jars are removed from the water bath after 30 minutes. The liquid extract from each jar is filtered by vacuum through two layers of filter paper in a Buchner funnel. The filtrate is collected in 500 mL glass filter flasks. The flask containing the filtrate is covered with foil while the second and third extractions are performed. Following the third extraction, the final volume of filtrate (composite of extracts 1, 2 and 3) is measured in a 500 mL graduated cylinder. The extracts are prepared for GC analysis by diluting 0.5 mL aliquots to 10 mL with benzene. The benzene is then dried with anhydrous sodium sulfate and loaded into GC autosampler vials (see note).

Inject 2 μL of the diluted extract into the GC column in duplicate.

Run standards singly at the beginning and end of each run.

Plot peak areas versus μg/L of standard injected to obtain standard curve for RDX.

6. CALCULATIONS

The concentration of explosive (ppm) in the sample is read directly from the standard curve. The apparent concentration of RDX in the compost is calculated from the following formula:

$$\text{concentration (ppm)} = \frac{\text{ppm} \times \text{total volume of extract (mL)} \times .001 \times \text{reciprocal of extract dilution}}{\text{20 g dry weight compost (50 g wet weight)}}$$

7. REFERENCE

Lindner, V. (1980), "Explosives and Propellants," Kirk-Othmer Encyclopedia Chemical Technology, 3rd edition, John Wiley and Sons, NY, 9:561-671.

8. NOTE: Several clean-up procedures were evaluated to remove compost interferences from RDX. These procedures either did not remove the interferences or also removed the RDX. Since the composting task was not for analytical methods development, it was decided to dilute the interferences out instead of spending a significant amount of additional time and monies in analytical methods development. As a result of the dilution, a high detection limit had to be accepted.

9. NOTE: Column clean-up of the acetone extracts of compost were investigated. Residual water was first removed from the acetone by pressing the acetone extract through anhydrous sodium sulfate. The dried extracts were passed through activated enutral alumina prior to GC analysis. Dried extracts were also passed through activated fluorasil and through Nuchar Attaclay in attempts at extract clean-up. Acetone extracts were also shaken with Nuchar Attaclay followed by centrifugation prior to analysis of the extract by GC.

Solvent exchange was investigated for sample clean-up by evaporating the acetone extract to dryness and redissolving the residue in methylene chloride. The methylene chloride extract was washed with water or 1 M HCl prior to analysis.

The use of N-P detector was also investigated. RDX detection limits using the N-P detector were similar to those of the FID. Interferences using the N-P detector were similar to those encountered with the electron capture detector.

RDX IN COMPOST

Target Concentration/Day	1	2	3	4
µg/g				
Blank 0	0	0	0	0
0.5X 630	528	756	566	731
X 1260	1562	1305	1202	1392
2X 2520	2904	2344	2359	2596
5X 6300	6593	5719	6020	6438
10X 12600	13008	12212	9701	9592

Target Concentration/Day	Average Found Value	Standard Deviation	Percent Imprecision	Percent Inaccuracy
µg/g				
Blank	0	0	0	0
0.5X 630	645.3	115.0	17.8	2.4
X 1260	1365.3	152.4	11.2	8.4
2X 2520	2550.8	262.3	10.3	1.2
5X 6300	6192.5	397.8	6.4	-1.7
10X 12600	11128.3			

USATHAMA DETECTION LIMIT PROOGRAM

RDX IN COMPOST

```
SUM(Y(i)=            43015.00
SUM(X(i)=            42840.00
SUM(X(i)↑2=       192099600.00
SUM(Y(i)↑2=       189324877.00
SUM(X(i)*Y(i)=       190269450.00
X=          1.0225 Y + -57.0771     CORR. COEF.=  0.9957
Y=          0.9780 X +  55.8228     CORR. COEF.=  0.9957
```

LINE	TC	C(FOUND)	C(CALC)	DELTA	GROUP
1	0.0000	0.0000	55.8228	-55.8228	0.0000
2	0.0000	0.0000	55.8228	-55.8228	0.0000
3	0.0000	0.0000	55.8228	-55.8228	0.0000
4	0.0000	0.0000	55.8228	-55.8228	0.0000
5	630.0000	528.0000	671.9778	-143.9778	1.0000
6	630.0000	756.0000	671.9778	84.0222	1.0000
7	630.0000	566.0000	671.9778	-105.9778	1.0000
8	630.0000	731.0000	671.9778	59.0222	1.0000
9	1260.0000	1562.0000	1288.1329	273.8671	2.0000
10	1260.0000	1305.0000	1288.1329	16.8671	2.0000
11	1260.0000	1202.0000	1288.1329	-86.1329	2.0000
12	1260.0000	1392.0000	1288.1329	103.8671	2.0000
13	2520.0000	2904.0000	2520.4430	383.5570	3.0000
14	2520.0000	2344.0000	2520.4430	-176.4430	3.0000
15	2520.0000	2359.0000	2520.4430	-161.4430	3.0000
16	2520.0000	2596.0000	2520.4430	75.5570	3.0000
17	6300.0000	6593.0000	6217.3734	375.6266	4.0000
18	6300.0000	5719.0000	6217.3734	-498.3734	4.0000
19	6300.0000	6020.0000	6217.3734	-197.3734	4.0000
20	6300.0000	6438.0000	6217.3734	220.6266	4.0000

```
.........................................
STANDARD  ERROR OF ESTIMATE (Sxy) =              215.46
N=                20.00   n=          1.00
TOTAL N=                20.00
t=                 1.73
t BASED ON TOTAL N
UPPER CONFIDENCE LIMIT AT(X=0) =                 446.90


upper confidence line at X=          630.00 is          1058.94
lower confidence line at X=          630.00 is           285.02
STANDARD DEVIATION AT X=             630.00  is          114.96
PERCENT INACCURACY AT X=             630.00  IS            2.42
PERCENT IMPRECISION AT X=            630.00  IS           17.82
MEAN FOUND AT  X=          630.00    IS          645.25

upper confidence line at X=         1260.00 is          1672.37
lower confidence line at X=         1260.00 is           903.89
STANDARD DEVIATION AT X=            1260.00  is          152.43
PERCENT INACCURACY AT X=            1260.00  IS            8.35
PERCENT IMPRECISION AT X=           1260.00  IS           11.17
MEAN FOUND AT  X=          1260.00   IS         1365.25

upper confidence line at X=         2520.00 is          2903.53
lower confidence line at X=         2520.00 is          2137.36
STANDARD DEVIATION AT X=            2520.00  is          262.26
PERCENT INACCURACY AT X=            2520.00  IS            1.32
PERCENT IMPRECISION AT X=           2520.00  IS           10.28
MEAN FOUND AT  X=          2520.00   IS         2550.75

upper confidence line at X=         6300.00 is          6630.42
lower confidence line at X=         6300.00 is          5804.33
STANDARD DEVIATION AT X=            6300.00  is          397.76
PERCENT INACCURACY AT X=            6300.00  IS           -1.71
PERCENT IMPRECISION AT X=           6300.00  IS            6.42
MEAN FOUND AT  X=          6300.00   IS         6192.50
```

DETECTION LIMIT = 794.65 ng/g

APPENDIX D. TEMPERATURE RECORDS FOR LABORATORY COMPOSTS

D-1. Laboratory Compost Temperature Records

Date	T4	T5	T6	T7	T8	T9	R4	R5	R6	R7	R8	R9	RC1	RC2	RC3	RC4	TC1	TC2	TC3	TC4	A	B
10/30	52	52	52	52	52	52	52	52	52	53	51	52	51	51	51	51	50	50	50	51	50	52
10/31	53	53	53	53	53	53	53	54	54	54	53	54	54	54	53	53	53	53	53	54	53	53
11/02	52	52	52	52	52	52	52	53	52	53	52	53	53	53	52	53	52	52	52	53	52	52
11/03	52	53	52	52	52	52	53	53	52	54	53	53	53	54	52	53	52	52	53	53	53	55
11/04	53	53	53	53	53	53	54	54	53	54	53	54	54	54	53	55	53	53	53	54	53	53
11/05	53	53	52	52	52	53	54	54	52	52	53	53	53	53	52	54	52	52	52	53	52	53
11/06	53	54	53	53	53	53	53	55	54	54	55	54	54	55	53	54	53	53	54	54	53	53
11/09	54	55	54	55	55	55	55	55	55	55	55	55	56	56	54	55	54	54	55	55	54	55
11/10	53	54	53	53	53	54	54	54	55	54	54	54	53	53	53	53	53	53	53	54	53	54
11/11	54	55	54	54	54	54	55	55	55	55	54	54	54	55	53	54	54	54	55	55	54	54
11/12	54	54	54	54	54	54	54	55	54	55	53	54	54	55	53	55	54	54	55	55	54	54
11/13	54	55	54	54	54	54	54	54	54	55	54	54	54	54	54	54	54	54	55	55	54	54
11/14	53	54	53	54	53	54	54	56	54	54	53	55	54	54	53	54	54	54	55	55	54	54
11/16	54	54	53	54	54	54	54	55	54	54	53	53	54	54	54	53	54	55	55	55	54	54
11/17	53	55	53	54	54	54	55	55	54	55	54	54	53	54	53	54	53	53	55	55	53	53
11/18	55	56	54	55		54	55	55	55	56	54	55	55	54	54	55	54	54	56	56	54	55
11/19	54	54	54	54	54	54	55	54	55	55	54	54	53	53	53	54	53	54	55	55	54	54

T4, T7, T8, R6, R7, R8, RC2, RC3, TC3, TC4 removed for analysis. Samples selected at random.

(continued)

(continued)

Date	T4	T5	T6	T7	T8	T9	R4	R5	R6	R7	R8	R9	RC1	RC2	RC3	RC4	TC1	TC2	TC3	TC4	A	B	C
11/20		59	54			54	55	58				56	55			56	55	55			55	55	
11/21		59	54			55	55	58				55	56			55	54	54			54	55	57
11/23		59	54			55	55	58				54	54			55	54	54			54	54	57
11/24		59	54			55	55	58				55	54			55	54	54			54	54	56
11/25		58	54			55	54	58				55	54			56	55	55			55	55	56
11/30		59	55			55	55	58				55	54			55	55	55			54	54	56
12/01		59	55			56	55	58				55	54			55	55	55			54	55	57
12/02		59	55			55	55	58				54	54			55	55	54			54	54	57
12/03		59	55			59	55	58				54	54			54	54	54			54	54	57
12/04		58	54			55	55	57				54	54			54	54	54			54	54	57
12/07		58	54			55	55	57				53	53			53	53	53			53	54	57
12/08		58	55			55	55	58				54	53			53	54	53			53	53	57
12/09		57	54			55	54	57				53	53			53	53	53			53	53	56
12/10		57	54			55	54	57				53	53			53	53	53			53	53	56

Samples extracted on 12/10. Six weeks incubation.

T4-9 TNT composts

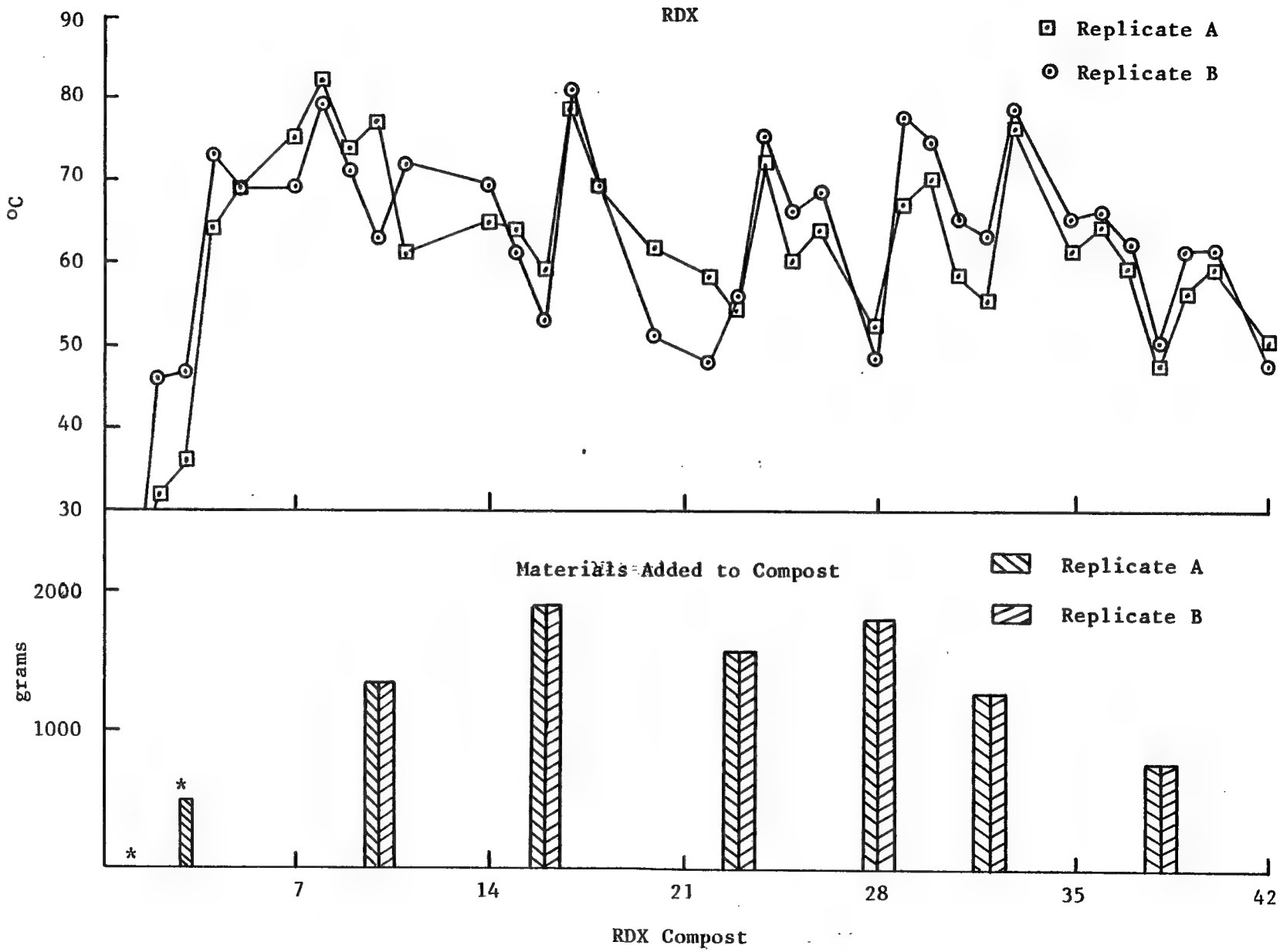
R4-9 RDX composts

RC1-4 RDX control composts

TC1-4 TNT control composts

A-C incubator temperature readings

APPENDIX E. TEMPERATURE RECORDS AND MATERIALS ADDED TO GREENHOUSE COMPOSTS



*Seed compost or manure added

Figure E-1. Temperature Profiles and Material Additions for RDX Greenhouse Composts

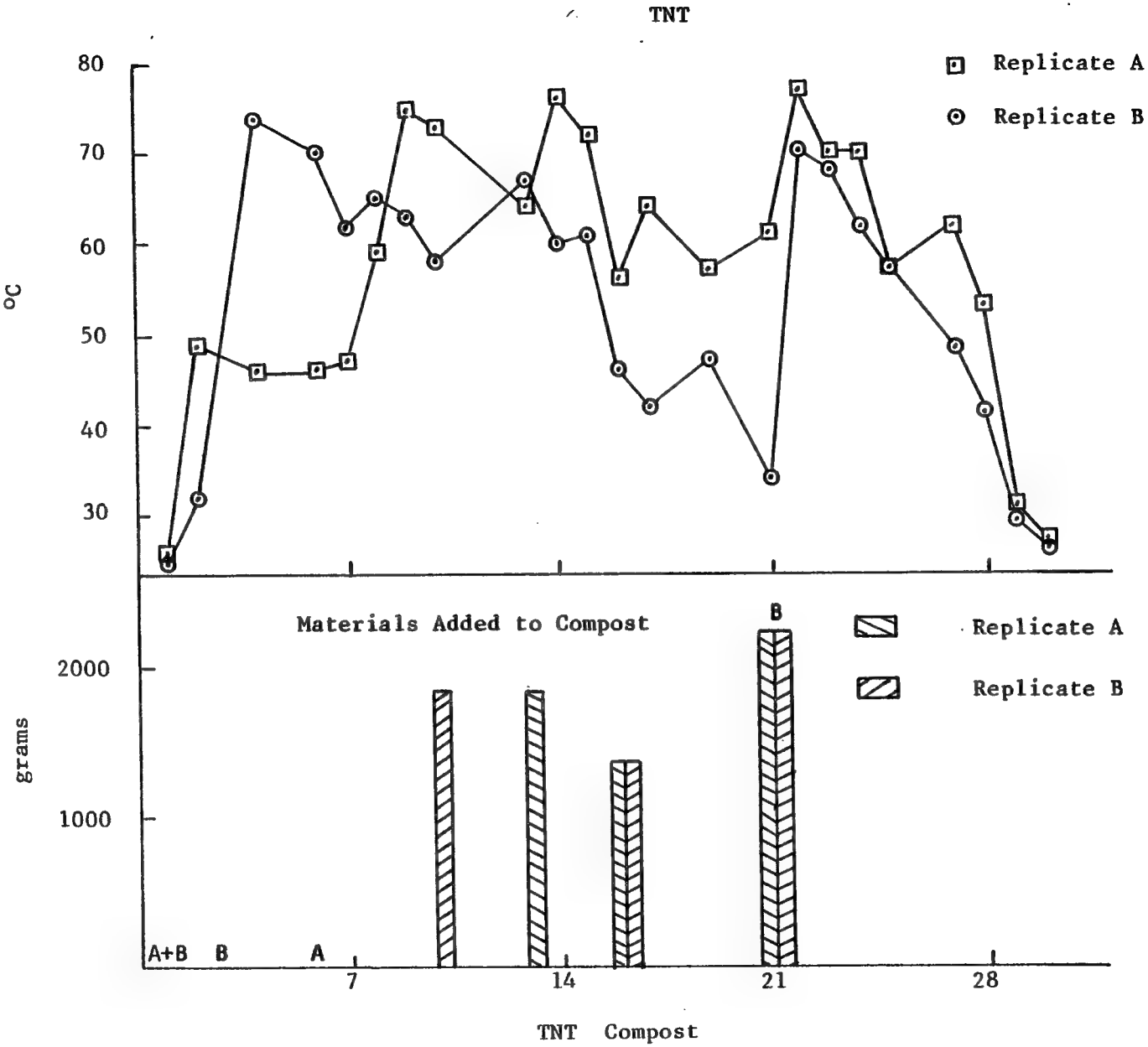
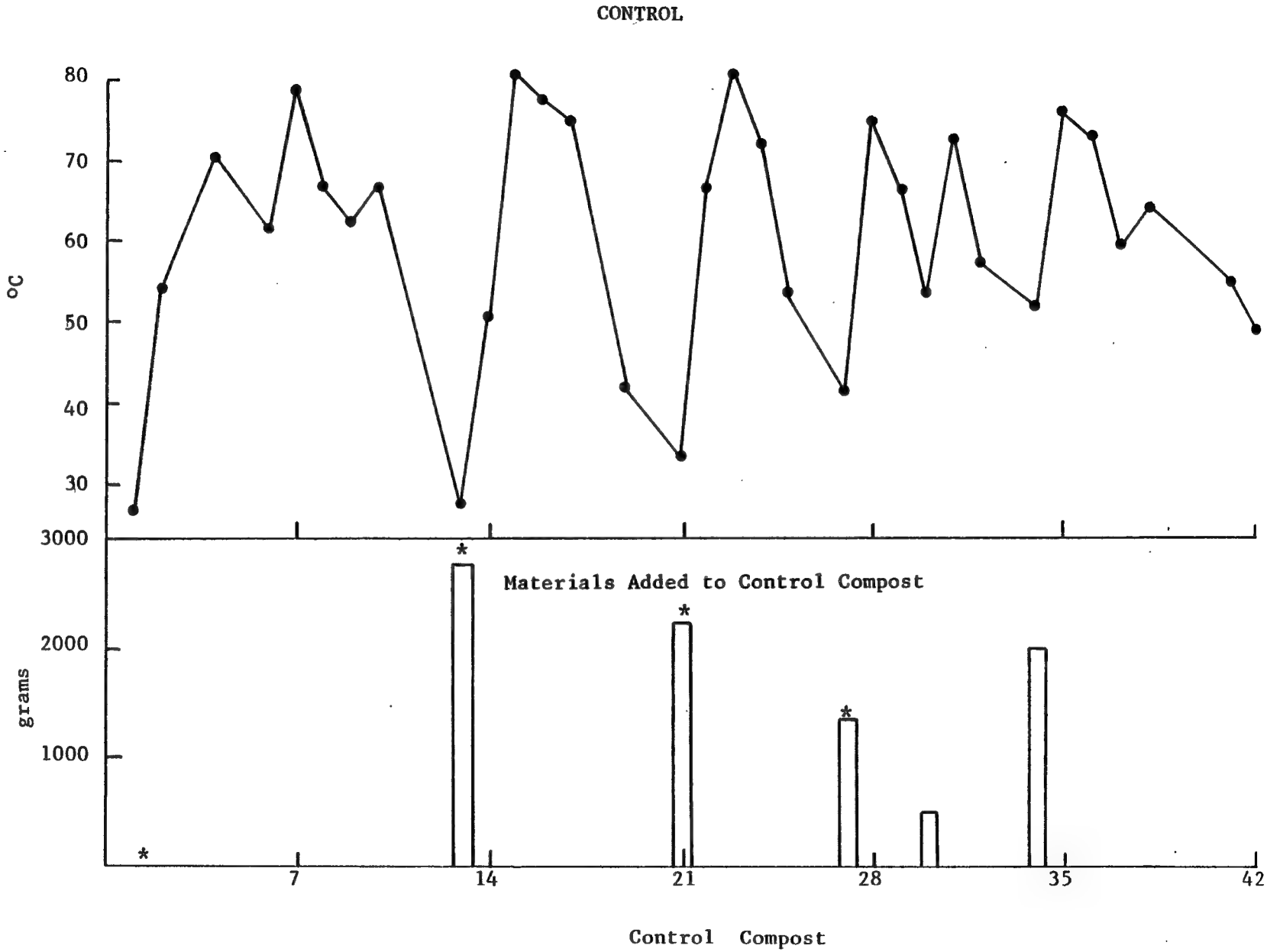


Figure E-2. Temperature Profiles and Materials Additions for TNT Greenhouse Composts



*Seed compost or manure added

Figure E-3. Temperature Profile and Materials Additions for Control Greenhouse Compost

APPENDIX F. ANALYSIS OF GREENHOUSE COMPOST ATMOSPHERES FOR OXYGEN AND CARBON DIOXIDE

Table F-1. Average Levels of O_2 and CO_2 in Greenhouse Compost Atmospheres

Compost	Length of Composting (days)	% O_2		% CO_2	
		$\bar{X}$	S	$\bar{X}$	S
Control	8	4.7		7.7	
	14	16.8		9.2	
	28	7.4		14.5	
	31	11.8		27.5	
	38	8.6		14.0	
TNT	8	14.4	4.4	4.0	5.7
	14	14.0	3.2	18.3	15.1
	28	19.3	4.3	3.1	4.4
RDX	4	13.6	0.5	14.7	4.0
	11	17.5	0.3	6.0	0.6
	17	14.9	4.9	14.9	8.3
	28	17.3	0.5	9.5	0.9
	32	16.5	<0.1	10.9	<0.1
	39	14.1	0.4	12.8	0.8

Average Levels of O_2 and CO_2 in Greenhouse Compost Atmospheres

APPENDIX G. PHOTOGRAPH OF A GREENHOUSE COMPOST

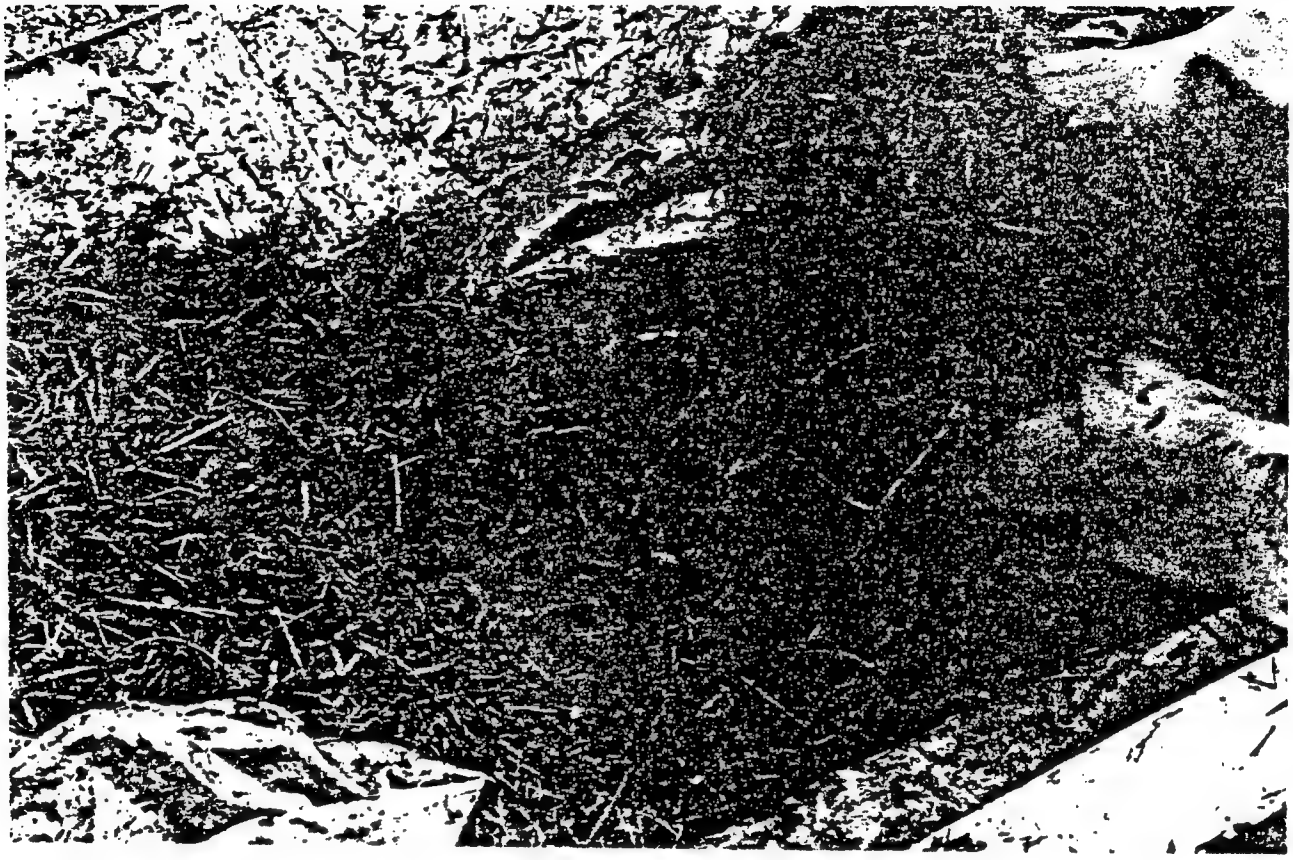


Figure G-1. Photograph of A Greenhouse Compost

LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	-	degrees centigrade
^{14}C	-	carbon 14; radioactive
^{14}C-RDX	-	uniformly ring labeled ^{14}C-RDX
^{14}C-TNT	-	uniformly ring labeled ^{14}C-TNT
CO_2	-	carbon dioxide
CPM	-	counts per minute
DNT	-	dinitrotoluene
DPM	-	disintegrations per minute
2-amino-DNT	-	2-amino-dinitrotoluene
4-amino-DNT	-	4-amino-dinitrotoluene
g	-	gram
GC	-	gas chromatograph
H_o	-	intersection of two quadratic equations for quench correction
H#	-	defines the relationship for the energy response of a specific sample to the energy response for an unquenched standard
H_2O	-	water
H_2SO_4	-	sulfuric acid
Kg	-	kilogram
LSC	-	liquid scintillation counter
μCi	-	microcurie
mCi	-	millicurie
µg	-	microgram
mg	-	milligram
mL	-	milliliter
μL	-	microliter
mm	-	millimeter
N	-	nitrogen
NaOH	-	sodium hydroxide
nm	-	nanometers
O_2	-	oxygen

pH	-	hydrogen ion concentration
RDX	-	hexahydro-1,3,5-trinitro-1,3,5-triazine
Rf	-	distance traveled relative to solvent front
tetra	-	tetra-nitroazoxytoluene
TLC	-	thin layer chromatography
2	-	2 sigma (95% confidence level)
UV	-	ultraviolet
w	-	week

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